U N I V E R S I T Y O F E D I N B U R G H

T H E S I S

entitled

"A STUDY OF THE VARIATIONS IN THE COMPOSITION OF CERTAIN HERBAGE SPECIES WITH SPECIAL RESPECT TO MAJOR ELEMENTS"

submitted

by

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INTRODUCTION

Pasture herbage is the gift of nature to cattle, sheep and horses. No other food can keep the herbivores in a better condition of health and productivity. But the productivity of the pasture and the nutritive value of the herbage play an important part in the resistance of the grazing animals to various diseases.

There is great advance in the breeding of grasses and a number of fertilisers are being used for their huge production. It seems the plant breeder and grassland agronomist have been creating problems for the animal husbandman, veterinary surgeon and the biochemist (Muir,1948). Lehr (1956) in his talk on the part played by fertilisation in establishing the mineral composition of grass pointed out that mineral composition can vary within very wide limits. Although it cannot yet be stated where the true line should be drawn, it is quite obvious that when a given constituent exceeds or falls short of a specific value difficulties are encountered either with the health or with the production of the cattle. Care must therefore be taken that the grass is provided with certain minimum fertiliser requirement and at the same time, that a certain balance is preserved between various constituents. It is a fact that any system of fertilisation which is unilaterally applied for a long period will eventually give rise to abnormal composition in either mineral or botanical composition of the grass.

Rye grass and white clover, the two most common and important grassland species, though widely used for the production/

production of meat and milk, sometimes cause disturbance to animal health. Though microelements may play a part, there is still much information to be unearthed about the major elements in pasture plants. One of the most common and fatal diseases to live stock due to a disturbing factor in the grass is hypomagnesaemic tetany. This tetany in ruminants, characterised by low levels of blood magnesium, has been reported from a number of countries, mostly where dairying is highly developed. It is difficult to judge whether its association with areas where quick maturing and high yielding stock are bred and the art of growing lush pastures from improved plant species by the application of artificial fertilisers, has been practised is causal or whether its apparent connection with these areas is the result of their more advanced veterinary diagnostic services. Walker-Love (1954-55) returning from his study tour in America commented "Herd sterility, bloat and milk fever crop up in all farming talk, but to me there was one thing which was conspicuous by its absence - grass staggers or tetany. Can this be related to less intensive manuring and management of grass?"

The disorder is usually called lactation tetany as it occurs most often in milking cows or grass tetany or grass staggers - because of its common occurrence in animals recently turned out to pasture in the spring. All these are in part misnomers, since the disorder occurs also, though less frequently in dry cows, bullocks, calves and breeding ewes and in/

in stall fed cattle receiving hay and concentrates as well as those on pastures.

Areas affected: This form of tetany has been reported in the Netherlands, Norway, England, Scotland, Ireland, America, New Zealand, Australia and Germany.

The incidence of the disease varies from year to year. In Norway it was recognised in about 1920 and appeared to increase as milk yield improved; during the second World War the incidence rose from three to five times the pre-war level (Brierem et al 1949). In the Netherlands on the contrary, it almost disappeared during the War (Sjollema 1951-52). The incidence is also seasonal. In the Netherlands, Britain, Germany and America the disease usually occurs in cattle and sheep turned out to graze on a lush growth of spring grass, although there is a second peak among the animals in the autumn (Sjollema 1932[1], Waringsholz, 1931) and outbreaks have occurred in stall fed cattle in winter (Blakemore and Stewart, 1933, Nicholson and Shearer, 1938 and Nolan and Hull 1941).

In New Zealand the disease occurs, usually between July and September, in cattle which are pastured throughout the year. The cows receive supplements of hay, silage, and sometimes roots during winter and spring and shortly after calving are transferred to fresh pasture which has not been grazed since being manured in the autumn (Hopkirk et al 1933). In Ireland the incidence occurs in cows whose sole food is pasture/

pasture in winter and which are never housed (Nicholson and Shearer 1938). In Queensland cows were put into a paddock of luscious, quickly growing feed for about a fortnight or month before the disorder appeared (Mulhearn, 1936). In Australia lush grazing produced by winter rains is associated with a high incidence of grass staggers in breeding ewes (Blumer et al, 1939). Incidence of blind staggers in horses occurring typically on lush protein feed, especially clover, young grasses or vetches was reported by Bruins(1947). In Scotland, though the disease has been causing much worry to flock masters, the worst year of incidence in sheep, both hill and low land, was 1951 when a large number of cases were reported and proved to be analogous to hypomagnesaemic tetany in cattle. The incidence in both hill and low ground ewes occurs at the same time of the year - April and May. The mortality is very high. A survey to-day would reveal that the disease is highly prevalent all over Scotland causing heavy losses both to stock breeders and dairy farmers (Stewart 1954).

Blakemore and Stewart (1933) confirmed hypomagnesaemia not only in clinical cases of tetany but in some apparently normal cows of the same herd at the same time. Similar observations of hypomagnesaemia in unaffected cows as well as in clinical cases, were made by Hopkirk et al,(1933) in New Zealand. Although low blood magnesium is the characteristic feature, Sjollema and Seekle (1930) reported marked/

marked hypocalcaemia as well as slightly subnormal occurrence of hypocalcaemia and in 1947 Allcroft showed that 76 percent of 406 cases of hypomagnesaemia was a concomitant hypocalcaemia. Blakemore and Stewart (1935) reported a seasonal drop in blood magnesium levels of cows put out to pastures in early spring without exhibitions of any clinical symptoms of tetany.

'T. Hart and Kemp (1956[1]) showed, by statistical investigation, that a significant correlation exists between the incidence of grass tetany and the 24 mean hour temperature. The spring cases almost all occurred between the time when the cattle went to grass and the date when the mean temperature rose above 14°C. In autumn few cows were affected until the mean temperature fell again to 14°C and after that cases continued to occur until the end of the pasture season. In a wet autumn the incidence was high and in spring the number of cows affected was greatest in years when the grass grew most quickly. During the summer months there were practically no tetany cases. This relation between temperature, weather and the incidence of grass tetany they related to the cationic composition of the herbage. Allcroft and Green (1938) and Allcroft (1947) found highest serum magnesium values in periods of high temperature and low magnesium levels were found at low temperature. Maximum values were found in summer months and the minimum values between December and April, the rate and extent of rise and

fall/

fall varying from year to year.

Green (1939) said that there were "grass tetany years" and years in which the incidence was almost negligible and that some times the disease seems to be associated with climatic variations which encourages sudden flush of grass growth but Allcroft (1947) found lowest serum magnesium levels in cold, wet and windy weather with temperatures below 42°F, little sun and little or no growth of grass.

Head and Rook (1955) suggest that hypomagnesaemia on grass and particularly during early spring arises from inadequate absorption of magnesium and is associated with ruminal ammonia production from excess of nitrogen from young spring grass. The production of abnormally high ammonia on feeding this grass was reflected by the presence of ammonia in the jugular blood. Derivaux (1953) reported that the pathogenesis of tetany was influenced not only by minerals but also be disturbance of protein metabolism.

'T. Hart and Kemp (1956[2]) studied the incidence of grass tetany on Dutch farms in 1954 and 1955 with a total of 3,942 cows. In cows on pastures dressed with 50 Kg. per hectare the incidence of tetany was 4.27 percent against 1.90 percent on pastures which received less than this amount or no nitrogenous dressing. Not only nitrogen, but also the potassium applied was concerned. The incidence was 0.52 percent on pastures low in potassium, 0.9 on pasture with sufficient and 5.20 percent with excess of potassium. When excess/

excess of potassium was combined with excess of nitrogen the incidence was 6.45 percent. Verdeyen (1953) reported that of 12 dairy cows on an experimental farm at Herve (Belgium) 6 had grass tetany in May 1948 when they were on pasture which had been dressed with potassium. In the previous year, when the protein content had been high and potassium lower, there had been no tetany.

Breirem et al (1949) produced hypomagnesaemia experimentally and found the curative dose for dairy cows was 20-25 grams of magnesium. Stewart and Reith (1956) gave an account of experiments suggesting that higher magnesium contents of herbage in April and May, produced by magnesian limestone dressings, considerably reduced the degree of hypomagnesaemia in a herd of cows where the disorder was prevalent. The results obtained by Bartlett et al (1954) support the idea of the magnesium content of the ration having an effect on the incidence of grass tetany.

Blakemore et al (1937) claimed that tetanic pastures have generally a higher manganese content than normal.

Kunkel et al (1953) showed that the feeding of potassium in the diet lowered the magnesium content of the serum significantly.

Workers in Texas (1954) lowered the magnesium level of sheep serum by the addition of potassium to a diet which by itself supported a normal level of serum magnesium.

Brouwer (1951,52) and Brouwer and Brandsma (1953) believe/

believe that the incidence of grass tetany is influenced by the ratio of K to Ca and Mg in the pasture. Verdeyen (1952) employs the ratio which he expresses as $\frac{K}{Ca + Mg}$, observing that grass tetany is liable to occur when the ratio of the pasture rises above 2.2.

Brouwer and his colleagues (Sjollema, 1951-52, Brouwer and Van de Vliert, 1951, Brouwer, 1951,52, Brouwer and Brandsina 1953) after studying a collection of analyses of Dutch pasture and common feeding stuffs showed that the pasture in which cattle develop acid urine with or without haemoglobinuria, show low alkali alkalinity (K + Na-Cl-S) and high alkaline earth alkalinity (Ca + Mg-P) and those in which grass tetany occurs show high alkali alkalinity and low alkaline earth alkalinity compared with normal pastures.

In Britain acetonomia was not often found associated with hypomagnesaemia in cattle but New Zealand workers have reported frequent association of the two conditions in dairy cows and have suggested that they may have a common cause (Swan 1948).

In the sixteen nation potash meeting at the International Potash Institute, London, in August 1956 (Farmer and Stock Breeder) vigorous discussion took place on the possible relation between grass tetany and the use of the potash fertilisers. Dr. E. Welte (Germany) while delivering his paper on "The significance of the potassium content of plants" said that he was not worried about the potash fertilisers in relation/

relation to grass tetany. But a Dutch speaker, who pointed out that the problem was serious in Holland, was inclined to disagree. A compatriot of Dr. Welte maintained that the disease had nothing to do with feeding. He maintained that it was purely a problem of constitution. Some animals were predisposed to a particular nervous irritation which resulted in grass tetany. In Normandy, a French representative pointed out, the best farmers insist that the composition of the pasture should be such that at least 25 percent is leguminous, then they have no trouble. He suggested that it was excessive use of nitrogenous fertilisers, virtually eliminating legumes, which created the Dutch problem.

Although it is more than 20 years since the first observations were made and many hypothesis have been advanced, the primary cause of hypomagnesaemia is not yet solved. Though Scotland has been experiencing serious losses from the incidence of the disease for a long time, extensive work has not yet been carried out towards the establishment of its primary causes. Even foreign workers (Kemp and 'T. Hart, 1957) commented on the lack of information on the mineral composition of Scottish pastures.

In Under the circumstances, it was thought advisable to carry out work on the variation of the mineral constituents of the grass under different seasons, weather conditions and treatment of different fertilisers.

MATERIALS AND METHODS

Effect of Nitrogen, Potassium and Clover on Rye Grass

Let N_1 and N_2 denote single and double levels of nitrogen respectively. Let K denote the presence of potassium and C that of clover.

The design selected has 6 blocks each containing 6 plots, a pair of blocks A and B forming a complete replication. The blocks are as follows:-

Ia	Ib	IIa	IIb	IIIa	IIIb
C	0	0	C	0	C
K	KC	KC	K	KC	K
N_1	N_1C	N_1C	N_1	N_1	N_1C
N_1KC	N_1K	N_1K	N_1KC	N_1KC	N_1K
N_2	N_2C	N_2	N_2C	N_2C	N_2
N_2KC	N_2K	N_2KC	N_2K	N_2K	N_2KC

The plots were randomised within each block. In this design the interaction of K C and N K C are partially confounded (with blocks). The loss of information is however slight.

Three blocks of land each of 16 yd. × 18yd. or 288 square yards were taken at the Bush House Demonstration Farm for the cultivation of grass. Each block was equally divided into 12 plots each of 24 square yards (3 × 8 yds). The soil analysis of the blocks is as follows:-

pH - 6.0

Exchangeable Ca - 0.082%

Available/

Available P_2O_5 (0.2N HCl extract) - 5 mg./100 g. soil (low)

Available K_2O (1% Citric acid extract) - 7 mg./100 g. soil (moderately low)

The actual layout of the plots is shown in this diagram.

Block 1

N_1	N_1KC	K	C	N_2KC	N_2
N_2C	N_1C	N_2K	KC	O	N_1K

Block 2

N_2KC	N_2	KC	O	N_1C	N_1K
N_1KC	N_2K	N_2C	K	C	N_1

Block 3

KC	N_2K	O	N_2C	N_1KC	N_1
N_1K	N_1C	K	N_2	N_2KC	C

Fertilisation:-

The fertilisers were applied on the 20th April, 1956 at the following rates, generally used by the farmers, per acre of grass land.

5 cwt. of superphosphate

3.75/

3.75 cwt. of potassium sulphate.

2.01 and 4.02 cwt. of ammonium nitrate (single and double dose) with respect to the nitrogen content, compared to 5 cwt. and 10 cwt. of nitrochalk used by the farmers.

The fertilisers, ammonium nitrate and potassium sulphate were pure salts. Superphosphate and potassium sulphate were applied once for the whole year. But the nitrogen fertiliser, ammonium nitrate, was divided between different cuts of the grass. As three cuts of grass are generally collected by the farmers in the course of a year, one third of the above mentioned dose of ammonium nitrate was applied for each cut.

Superphosphate was applied to all the plots, each plot of 24 square yards received 1261.0 grams of the fertiliser. The potassium plots of 24 square yards each received 946 grams of potassium sulphate. The plots of 24 square yards, for single and double level of nitrogen, received 186 grams and 372 grams of ammonium nitrate respectively.

The grasses selected were Perennial Ryegrass, S100 and white clover, S24. The seed rate for Ryegrass was 30 pounds per acre. The inclusion of clover was at the rate of 2 pounds per acre.

On the 15th May, the seeds were broadcast and to ensure even distribution they were mixed with sand. The soil being freshly cultivated there was a heavy infestation with docks and chickweed. The weeds threatened to suppress the growth of grass and the plots had to be cleaned out by hand./

hand. Very little clover was present in the plots receiving double dose of nitrogen with or without potassium. When the grass was about 6 inches high it was cut on the 18th July, 1956. Ammonium nitrate was applied on the 20th July and the second cut collected on the 20th August, 1956. As the grass could not be grown earlier in 1956 only two cuts were collected.

The potassium fertiliser was applied on the 23rd December, 1956 and ammonium nitrate on the 7th March, 1957. The cut for 1957 was taken on the 23rd of May, 1957.

A representative sample of about a pound was taken from each plot. This sample was mixed and sub-divided by quartering till the required amount was obtained. The sample was dipped in a sink of water to get rid of soil contamination and other external materials and the weeds were picked out. The whole process was completed within a minute or two to avoid leaching of mineral matter. The sample was spread on a piece of wire netting to drain off the water and was then dried in an air oven for about 24 hours at a temperature of 100°C. The dry material was finely ground in a mill and bottled for analysis.

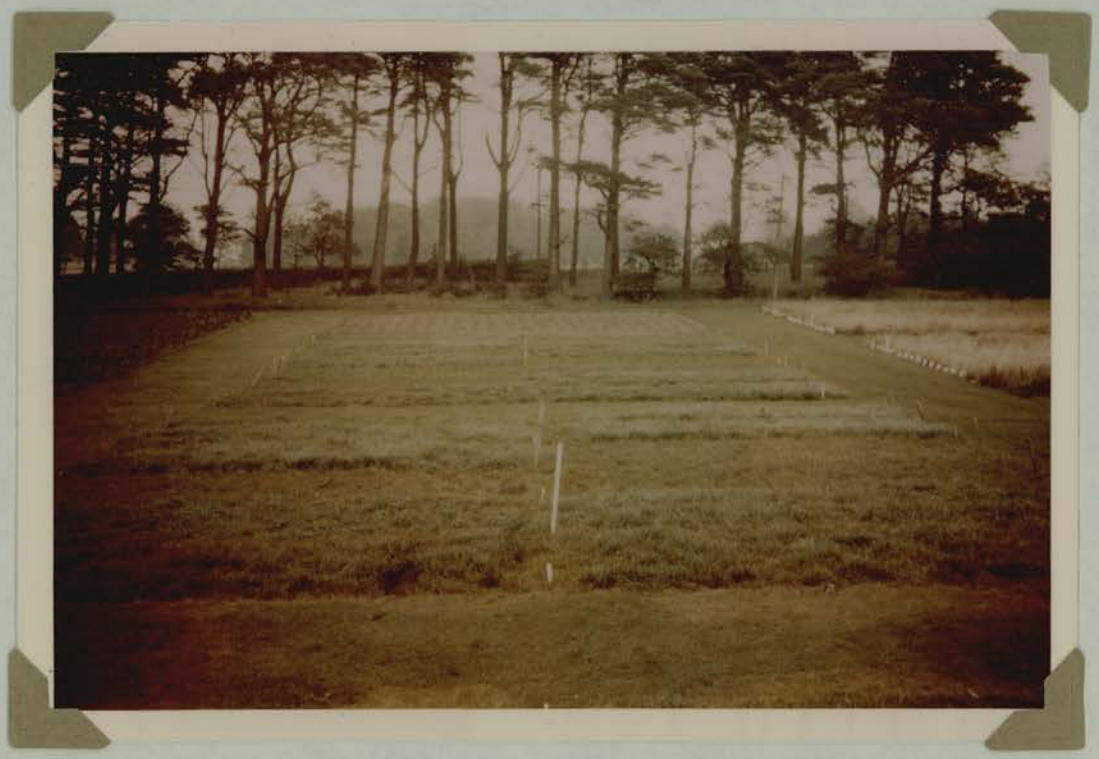
The methods of analysis of the various elements are described in the appendix. The reproducibility of the different methods are also shown in the appendix.

RESULTS

Plate 1

Layout of Plot 1

Plate 2



Layout of Plots 1 and 2

The growth and the yield of grass varied proportionally to the different levels of nitrogen fertilisers applied as can be seen from Table I below. The layouts of the plots and the contrast of growth in the grass with the different fertilisers can be judged from the three plates 1, 2, and 3 (Plates 1 and 2 opposite page 14 and plate 3 opposite page 15). Higher levels of nitrogen fertiliser almost eliminated the clover population and can be seen in plate 4 (opposite page 15). Plate 5 (opposite page 16) shows the contrast between the treatments of N_2C and N_2K.

Table I

Average yield of grass, on green matter basis, of each subplot of 24 sq.yd. (3 x 8) under different treatments

Treatments	First cut on 18.7.56	Second cut on 20.8.56	Third cut on 23.5.57
	lb.	lb.	lb.
O	1.3	2.6	6.6
K	2.1	2.2	5.3
C	4.9	7.5	14.5
KC	4.5	7.2	12.1
N_1	6.7	12.3	16.0
N_1C	7.8	18.8	21.8
N_1K	5.7	10.7	15.9
N_1KC	6.2	16.3	22.1
N_2	11.5	27.0	29.8
N_2C	12.0	30.0	29.9
N_2K	9.8	24.8	30.2
N_2KC	14.5	31.7	34.6

The chemical composition of the replicated samples of grass and method of statistical calculation are shown in the Appendix.

In all the three cuts of grasses the terms under the statistical calculations are as follows:-

Plate 3

Layout of Plots 1, 2 and 3

Plate 4

N_2KC C

Under Main N, N_0 represents 12 plots of grasses having no nitrogen fertiliser, N_1 represents 12 plots of grasses treated with a single dose of nitrogen fertiliser and N_2 represents the grass in the 12 plots having a double dose of nitrogen fertiliser.

Under main K, K_0, represents 18 plots of grasses having received no potassium fertiliser and K represents the 18 plots of grasses with potassium fertiliser.

Under main C, C_0 represents 18 plots of grasses having no clover with them and C represents the 18 grasses having clover growth with them.

Under inter NK, each of N_0K_0, N_1K_0, N_2K_0, N_0K, N_1K and N_2K represents 6 plots of grasses. N_0K_0 represents the grasses in the plots where there was no nitrogen or potassium fertilisers. N_1K_0 represents the grasses which received a single dose of nitrogen but no potassium, N_2K_0 are those grasses which had a double dose of nitrogen fertiliser without potassium. N_0K refers to the grasses which were treated with potassium fertiliser but no nitrogen. N_1K represents the grasses having a single level of nitrogen with potassium fertiliser and N_2K represents the grasses having a double level of nitrogen with potassium.

Under NC interaction, the above paragraph applies replacing K with C.

Under inter KC, K_0C_0, K_0C, KC_0 and KC each represents 9 plots.

Results of First cut (18.7.56).

Table II shows the chemical composition of the grass (mean value) under different treatment.

Plate 5

N_2K N_2C

Table II

1956 FIRST CUT (date?)

Chemical composition of grass under different treatments on dry matter basis (mean value)

Treatments	Total N %	Protein N %	Ash %	Silica %	Ca %	Mg %	Cl %	P %	S %	Na %	K %	Mn p.p.m.
Control	1.44	1.31	11.53	2.80	0.55	0.23	0.41	0.40	0.43	0.08	3.36	111.0
K	1.52	1.28	9.31	2.51	0.50	0.15	0.77	0.41	0.28	0.08	3.00	135.0
C	1.83	1.39	10.05	2.39	0.56	0.19	0.52	0.30	0.35	0.08	3.36	141.0
KC	1.99	1.76	9.95	1.96	0.47	0.15	0.41	0.37	0.31	0.13	3.78	109.0
N_1	1.60	1.32	8.57	1.61	0.53	0.17	0.37	0.30	0.29	0.13	2.72	165.0
N_1C	1.78	1.30	9.97	2.67	0.51	0.18	0.37	0.36	0.26	0.13	2.99	143.0
N_1K	1.90	1.49	9.04	1.89	0.46	0.16	0.44	0.37	0.34	0.13	2.99	119.0
N_1KC	1.80	1.26	9.73	2.57	0.47	0.17	0.41	0.32	0.29	0.08	3.23	110.0
N_2	2.31	1.67	9.38	2.21	0.47	0.17	0.35	0.33	0.41	0.17	3.24	129.0
N_2C	2.10	1.63	9.55	1.70	0.52	0.18	0.40	0.36	0.30	0.27	2.99	126.0
N_2K	2.22	1.76	10.40	2.58	0.46	0.18	0.48	0.35	0.30	0.20	3.76	86.0
N_2KC	1.94	1.29	9.84	1.87	0.44	0.16	0.53	0.36	0.34	0.14	3.50	111.0

The statistical results of each individual element are shown in the next few pages.

Statistical Results.

Nitrogen:-

Significant difference -

Main N -

$N_1 > N_0$ (5%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%).

Main K - Not significant.

Main C -

$C > C_0$ (1%)

Inter NK -

$N_2K_0 > N_0K_0$ (1%), $N_2K_0 > N_1K_0$ (1%)

$N_2K > N_0K$ (1%), $N_2K > N_1K$ (1%)

$N_0K > N_0K_0$ (5%), $N_1K > N_1K_0$ (1%), $N_2K_0 > N_2K$ (5%)

Inter KC - Not significant.

Inter NC-

$N_1C_0 > N_0C_0$ (1%), $N_2C_0 > N_0C_0$ (1%), $N_2C_0 > N_1C_0$ (1%)

$N_0C > N_1C$ (5%), $N_2C > N_1C$ (1%)

$N_0C > N_0C_0$ (1%), $N_2C_0 > N_2C$ (1%)

—— oOo ——

Protein Nitrogen:-

Significant difference -

Main N -

$N_0 > N_1$ (5%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K/

Main K - Not significant.

Main C - Not significant.

Inter NK -

$N_2K_0 > N_0K_0$ (1%), $N_2K_0 > N_1K_0$ (1%)

$N_0K > N_1K$ (5%), $N_2K > N_1K$ (1%)

$N_0K > N_0K_0$ (1%), $N_0K > N_1K_0$ (1%)

$N_2K_0 > N_2K$ (5%)

$N_2K_0 > N_1K$ (1%), $N_2K_0 > N_0K$ (1%)

Inter KC - Not significant.

Inter NC -

$N_2C_0 > N_0C_0$ (1%), $N_2C_0 > N_1C_0$ (1%), $N_1C_0 > N_0C_0$ (5%)

$N_0C > N_1C$ (1%), $N_0C > N_2C$ (5%), $N_2C > N_1C$ (1%)

$N_0C > N_0C_0$ (1%), $N_0C > N_1C_0$ (1%), $N_1C_0 > N_1C$ (5%)

$N_2C_0 > N_oC$ (1%), $N_2C_0 > N_1C$ (1%), $N_2C_0 > N_2C$ (1%)

——— oOo ———

Calcium:-

Significant difference -

Main N -

$N_0 > N_1$ (5%), $N_0 > N_2$ (1%).

Main K -

$K_0 > K$ (1%).

Main C - Not significant.

Inter NK - Not significant.

Inter KC - Not significant.

Inter NC - Not significant.

——— oOo ———

Magnesium:-/

Magnesium:-

Significant difference -

Main N -

$N_0 > N_1$ (1%)

Main K -

$K_0 > K$ (1%)

Main C - Not significant.

Inter NK -

$N_0K_0 > N_1K_0$ (1%), $N_0K_0 > N_2K_0$ (1%)

$N_0K_0 > N_0K$ (1%), $N_0K_0 > N_1K$ (1%), $N_0K_0 > N_2K$ (1%)

$N_1K_0 > N_0K$ (1%), $N_2K_0 > N_0K$ (1%), $N_2K > N_0K$ (1%)

Inter KC - Not significant.

Inter NC -

$N_0C_0 > N_1C_0$ (1%), $N_0C_0 > N_2C_0$ (1%).

$N_0C_0 > N_0C$ (1%), $N_0C_0 > N_1C$ (1%), $N_0C_0 > N_2C$ (1%)

——— oOo ———

Chlorine:-

Significant difference -

Main N -

$N_0 > N_1$ (1%), $N_0 > N_2$ (1%), $N_2 > N_1$ (5%)

Main K -

$K > K_0$ (1%)

Main C - Not significant.

Inter NK - Not significant.

Inter KC/

Inter KC -

$KC_0 > K_0C_0$ (1%), $KC_0 > KC$ (1%), $KC_0 > K_0C$ (1%)

$K_0C > K_0C_0$ (1%), $KC > K_0C_0$ (1%)

Inter NC -

$N_0C_0 > N_1C_0$ (1%), $N_0C_0 > N_2C_0$ (1%)

$N_0C > N_1C$ (1%), $N_0C_0 > N_0C$ (1%), $N_0C_0 > N_1C$ (1%).

$N_0C_0 > N_2C$ (1%), $N_2C > N_1C$ (1%), $N_2C > N_1C_0$ (5%)

—— oOo ——

Phosphorus:-

Significant difference -

Main N -

$N_0 > N_1$ (1%), $N_0 > N_2$ (5%)

Main K -

$K > K_0$ (1%)

Main C -

$C_0 > C$ (5%)

Inter NK - Not significant.

Inter KC - Not significant.

Inter NC -

$N_0C_0 > N_1C_0$ (1%), $N_0C_0 > N_2C_0$ (1%)

$N_0C_0 > N_0C$ (1%), $N_0C_0 > N_1C$ (1%), $N_0C_0 > N_2C$ (1%)

—— oOo ——

Sulphur:-/

Sulphur:-

Significant difference -

Main N -

$N_0 > N_1$ (1%), $N_2 > N_1$ (1%)

Main K -

$K_0 > K$ (1%)

Main C -

$C_0 > C$ (1%)

Inter NK -

$N_0K_0 > N_1K_0$ (1%), $N_2K_0 > N_1KO$ (1%)

$N_0K_0 > N_0K$ (1%), $N_0K_0 > N_1K$ (1%), $N_0K_0 > N_2K$ (1%)

$N_2K > N_0K$ (5%),

$N_1K > N_1KO$ (1%), $N_2K_0 > N_2K$ (5%)

Inter KC -

$K_0C_0 > KC_0$ (1%), $K_0C_0 > K_0C$ (1%)

$K_0C_0 > K_C$ (1%)

Inter NC -

Not significant.

—— oOo ——

Sodium:-

Significant difference -

Main N -

$N_2 > N_1$ (1%), $N_2 > N_0$ (1%)

Main K/

Main K - Not significant.

Main C - Not significant.

Inter NK -

$N_1K_0 > N_0K_0$ (1%), $N_2K_0 > N_0K_0$ (1%), $N_2K_0 > N_1K_0$ (1%)
$N_2K > N_1K$ (1%), $N_2K > N_0K$ (1%), $N_2K_0 > N_2K$ (1%)

Inter KC -

$K_0C > KC$ (1%), $K_0C > K_0C_0$ (5%)

Inter NC - Not significant.

——— oOo ———

Potassium:-

Significant difference -

Main N -

$N_0 > N_1$ (1%), $N_2 > N_1$ (1%)

Main K -

$K > K_0$ (5%)

Main C -

$C > C_0$ (5%)

Inter NK -

$N_0K_0 > N_1K_0$ (1%), $N_0K > N_1K_0$ (1%)
$N_2K > N_0K$ (1%), $N_2K > N_1K$ (1%)
$N_2K > N_2K_0$ (1%), $N_2K > N_1K_0$ (1%)

Inter NC -

$N_2C_0 > N_0K_0$ (1%), $N_2C_0 > N_1C_0$ (1%)
$N_0C > N_0C_0$ (1%), $N_0C > N_1C$ (1%), $N_0C > N_2C$ (5%)
$N_2C_0 > N_1C$ (1%)

Inter KC -

$KC > K_0C$ (1%), $KC > KC_0$ (1%), $KC > K_0C_0$ (1%)

——— oOo ———

Manganese:-

Significant difference -

Main N - Not significant.

Main K -

$K_0 > K$ (1%)

Main C - Not significant.

Inter NK - Not significant.

Inter KC -

$K_0C > KC$ (1%), $K_0C_0 > KC$ (1%)

$K_0C > KC_0$(5%), $K_0C_0 > KC_0$ (5%).

Inter NC - Not significant.

——— oOo ———

Discussion

The total nitrogen content of the grass increased under all the fertiliser treatments and the increment ranged from 5.5 to 60.4%. Under the treatment of nitrogen fertiliser, the increment was proportional to the dose applied. The grass having clover without nitrogen helped the increment better than those grasses having a single dose of nitrogen with clover. In the double level nitrogen groups the grasses having no clover had more nitrogen than those grasses having clover with them, although most of the clovers were eliminated by the heavy dressing of the fertiliser. It seems that clover in presence of a higher dose of nitrogen reduces the uptake of nitrogen by the grass.

In the combined effect of nitrogen and potassium fertilisers, a single dose of nitrogen with potassium increased the nitrogen content of the grass better than a single dose without potassium. But in the case of the double dose the reverse was the case, the increment of nitrogen being better in the grasses having a double dose without potassium than in those having the same dose with potassium.

Grasses having no nitrogen fertiliser contained more protein nitrogen than those which received a single dose of nitrogen, but the protein nitrogen increased with the application of a double dose of the fertiliser. The increment was up to 34.3%. The grasses having potassium fertiliser without nitrogen had a better protein nitrogen content/

content than those receiving a single dose of nitrogen fertiliser. But in the case of the double dose the increment was higher when the grasses had no potassium fertiliser treatment. The grasses having clover in them without nitrogen fertiliser had more protein nitrogen than those which received a single or a double dose of nitrogen in the clover. In the case of the double dose of nitrogen fertiliser, the addition of clover with the grass led to a smaller amount of protein nitrogen than in those having no clover.

Ca ? The nitrogen fertiliser had an adverse effect on the calcium metabolism in the grass and the decrease was proportional to the level of fertiliser applied. The potassium fertiliser also decreased the calcium content of the grass. The maximum loss of calcium by the grass under the treatment of N_2KC was 20%.

Mg The magnesium content of the grass decreased under all the treatments. The maximum decrease was up to 35%. The grasses having a single dose of nitrogen fertiliser had less magnesium than those having no treatment. The double dose of nitrogen slightly improved the situation. The groups of grass that received the potassium fertiliser had less magnesium than those having no potassium fertiliser. Addition of nitrogen with potassium fertiliser slightly improved the magnesium metabolism in grass when compared to the grasses having potassium but no nitrogen fertiliser. Combination of nitrogen fertiliser with clover decreased the magnesium content of rye grass when compared with the grasses that received no nitrogen and no clover.

Nitrogen/

Cl

Nitrogen fertiliser decreased the chlorine content of grass but the decrease was less with the double dose than the single dose. Although potassium as a fertiliser was used in the form of potassium sulphate, the chlorine content of grasses, treated with this, increased by about 88%. The combined effect of potassium and clover improved the chlorine metabolism in the grass when compared with grass that received no clover and no potassium and grasses with potassium but without clover had a better chlorine content. Combination of nitrogen with clover decreased the chlorine content of grasses when compared with those that received no nitrogen and without clover, But in the combination the decrease of the element was more in the single dose than the double dose of nitrogen.

P

The effect of potassium was to increase the phosphorus content of grass. The nitrogen fertiliser, on the other hand decreased the element in the grass. Both the single and the double dose of nitrogen fertiliser decreased the phosphorus content of grass. The presence of clover to the grass also decreased the phosphorus content of the grass. The combined effect of nitrogen and clover also decreased the phosphorus in the grass.

S

The sulphur content of the grasses was decreased by all the treatments. The double dose of nitrogen fertiliser had less effect than the single dose on the sulphur content. Though potassium was applied as potassium sulphate, this application, too, decreased the sulphur content of the grass.

Inclusion/

Inclusion of clover in the grass also had a bad effect on the uptake of the element by the grass. The combined effect of nitrogen and potassium was to decrease the sulphur in the grass, but not so much as the single dose of nitrogen without potassium. But when the grasses received a double dose of nitrogen without potassium the decrease was less than when the double dose was combined with potassium. The combination of potassium with clover had the greatest effect on the grass with respect to depressing its uptake of sulphur.

Na

The application of nitrogen fertiliser increased the sodium content, the double dose increasing it more than the single dose. The greatest increase of the element was 237%. In the increment of this element by the combined effect of nitrogen and potassium, a double dose of nitrogen with potassium increased the sodium more than a single dose with potassium. A double dose of nitrogen without potassium increased the sodium in the grass more than a double dose in the potassium. The grasses having clover without potassium had more sodium than the grass with both clover and potassium or with neither clover nor potassium.

K

The content of potassium was increased or decreased under different treatments. All grasses having either a double dose of nitrogen fertiliser or no nitrogen fertiliser had more potassium than those having a single dose of nitrogen. Both potassium and clover tended to increase the potassium in the grass. In the combined effect of nitrogen and potassium a double dose of nitrogen showed the greater effect in increasing the potassium in the grass. The combination of nitrogen/

nitrogen in the clover decreased the potassium in the grass. The grasses having clover without nitrogen had more potassium than those having a single or a double dose of nitrogen with clover. The combination of potassium with clover increased the potassium content of the grass when compared with potassium without clover or clover without potassium. As double dose of nitrogen was favourable than single dose, the grasses that had a double dose of nitrogen with potassium and with clover tended to have increasing amounts of potassium.

Mn

Potassium fertiliser tended in general to decrease the manganese content of grass. The combined effect of clover and potassium fertiliser was also to decrease the manganese content of the grass.

Elements generally act in an ionic state and they don't act independently in the animal system but diverse minerals may exert either similar or opposite effects. This knowledge and the early works of some Hungarian investigators (Hutyra et al 1938, Marek et al, 1931 and Wellmann, 1931) prompted Brouwer et al (1951) to develop new methods in the study of the behaviour of minerals in combination. By an efficacious combination of a number of minerals they calculated some specific magnitudes. The following are the magnitudes calculated after the mineral contents of the grass had been calculated as milli equivalents per Kg. of dry matter.

AA = Alkali Alkalinity = K + Na - Cl - S

EA/

EA = Alkaline Earth Alkalinity = Ca +Mg - P

TA = AA + EA = Total Alkalinity = Base Excess

VA = AA - EA = Alkalinity difference.

It was thought worth while to calculate the mineral contents of the grass, under different treatments, in the same manner and see how the same grass behaves under the treatments.

Table III on page 30 shows the figures as milli equivalents per Kg. of dry matter.

The lowest figure for EA are obtained in the grass treated with potassium fertiliser and the highest figure for AA was found in the grass given a double dose of nitrogen and potassium fertiliser. These are the conditions found in grass tetany according to Brouwer and his colleagues (Sjollema, 1951-52, Brouwer and Van de Vliert 1951, Brouwer 1951, 1952, Brouwer and Brandsma 1953) who showed that grass from tetany inducing areas was characterised by extremely high Alkali Alkalinity (AA) and extremely low Alkaline Earth - Alkalinity (EA). compared to normal grasses.

Result of Second cut/

Table III

Milli equivalents per Kg of dry matter (P = 3 equivalents)

(1st cut)

Treatment	AA Alkali Alkalinity K+Na-Cl-S	EA Alkaline Earth Alkalinity Ca+Mg-P	TA = AA+EA Total Alkalinity	VA = AA-EA Base Excess	$\frac{K}{Ca + Mg}$
Control	491.0	76.0	567.0	415.0	1.86
K	410.0	- 24.0	386.0	434.0	2.06
C	529.0	145.0	674.0	384.0	1.97
KC	525.0	0.0	525.0	525.0	2.70
N_1	468.0	114.0	582.0	354.0	1.72
N_1C	556.0	54.0	610.0	502.0	1.90
N_1K	486.0	4.0	490.0	482.0	2.11
N_1KC	564.0	66.0	630.0	498.0	2.20
N_2	548.0	56.0	604.0	492.0	2.21
N_2C	582.0	59.0	642.0	523.0	1.88
N_2K	727.0	40.0	767.0	687.0	2.55
N_2KC	496.0	4.0	500.0	492.0	2.54

Result of Second cut (20.8.56).

Table IV

Chemical composition of grass under different treatments on dry matter basis (mean value)

Treatments	Total N %	Protein N %	Ash %	Silica %	Ca %	Mg %	Cl %	P %	S %	Na %	K %	Mn p.p.m.
Control	2.16	1.56	11.25	2.77	0.47	0.20	0.46	0.45	0.52	0.05	3.41	139.0
K	1.84	1.44	10.33	1.83	0.46	0.20	0.58	0.37	0.41	0.05	3.47	161.0
C	2.86	1.47	11.70	1.96	0.59	0.23	0.48	0.48	0.43	0.07	4.20	138.0
KC	2.64	1.61	11.07	1.97	0.49	0.22	0.62	0.43	0.44	0.05	3.88	139.0
N_1	2.85	1.37	11.37	1.62	0.55	0.21	0.38	0.43	0.39	0.14	4.84	102.0
N_1C	2.98	1.39	11.53	1.62	0.53	0.22	0.48	0.53	0.46	0.13	4.37	93.0
N_1K	2.78	1.33	10.93	1.50	0.45	0.19	0.54	0.43	0.38	0.07	3.99	101.0
N_1KC	2.82	1.42	11.89	1.47	0.50	0.24	0.45	0.45	0.40	0.06	4.78	99.0
N_2	3.42	1.85	11.26	1.41	0.54	0.22	0.34	0.51	0.40	0.19	4.41	83.0
N_2C	3.15	1.52	11.01	1.64	0.51	0.23	0.43	0.49	0.38	0.22	4.18	127.0
N_2K	2.95	1.62	11.26	1.63	0.54	0.21	0.37	0.46	0.41	0.15	4.94	116.0
N_2KC	3.23	1.92	12.68	1.53	0.44	0.21	0.45	0.53	0.43	0.13	5.29	109.0

The next few pages show the statistical results of the main action and interaction of different fertilisers on individual elements in the grass.

Statistical Results.

Total Nitrogen:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K -

$K_0 > K$ (1%)

Main C -

$C > C_0$ (1%)

Inter NK - Not significant.

Inter KC -

$K_0C > K_0C_0$ (1%), $K_0C > KC_0$ (1%)

$KC > KC_0$ (1%)

Inter NC -

$N_2C > N_1C$ (1%), $N_2C > N_0C$ (1%)

$N_0C > N_0C_0$ (1%), $N_1C_0 > N_0C_0$ (1%),

$N_2C_0 > N_1C_0$ (1%), $N_2C_0 > N_0C_0$ (1%),

$N_2C_0 > N_0C$ (1%), $N_2C_0 > N_1C$ (1%),

$N_1C > N_0C_0$ (1%), $N_1C_0 > N_0C_0$ (1%)

—— oOo ——

Protein Nitrogen:- /

Protein Nitrogen:-

Significant difference -

Main N -

$N_0 > N_1$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K - Not significant.

Main C - Not significant.

Inter NK - Not significant.

Inter KC -

$KC > K_0C$ (1%), $KC > KC_0$ (1%)

Inter NC - Not significant.

——— oOo ———

Calcium:-

Significant difference -

Main N - Not significant.

Main K -

$K_0 > K$ (1%)

Main C - Not significant.

Inter NK - Not significant.

Inter KC - Not significant.

Inter NC -

$N_0C > N_1C$ (1%), $N_0C > N_2C$ (1%), $N_1C > N_2C$ (1%)

$N_0C > N_0C_0$ (1%), $N_2C_0 > N_1C_0$(1%),

$N_2C_0 > N_2C$ (1%), $N_2C_0 > N_1C$ (5%), $N_1C_0 > N_0C_0$ (1%)

——— oOo ———

Magnesium:-/

Magnesium:-

Significant difference -

Main N - Not significant.

Main K - Not significant.

Main C -

$C > C_0$ (1%)

Inter NK - Not significant.

Inter KC - Not significant.

Inter NC -

$N_0C > N_0C_0$ (1%), $N_0C > N_1C_0$ (1%)

$N_1C > N_0C_0$ (1%), $N_1C > N_1C_0$ (1%)

$N_2C > N_0C_0$ (5%), $N_2C > N_1C_0$ (5%)

——— oOo ———

Chlorine:-

Significant difference -

Main N -

$N_0 > N_1$ (1%), $N_0 > N_2$ (1%), $N_1 > N_2$ (1%)

Main K -

$K > K_0$ (1%)

Main C -

$C > C_0$ (1%)

Inter NK - not significant.

Inter KC -

$K_0C > K_0C_0$ (1%), $KC > K_0C$ (1%)

$KC > K_0C_0$ (1%), $KC_0 > K_0C_0$ (1%)

$KC_0 > K_0C$ (1%)

Inter NC-/

Inter NC -

$N_0C > N_1C$ (1%), $N_0C > N_2C$ (1%)
$N_1C > N_2C$ (1%), $N_0C_0 > N_1C_0$ (1%)
$N_0C_0 > N_2C_0$ (1%), $N_1C_0 > N_2C_0$ (1%)
$N_0C > N_1C_0$ (1%), $N_0C > N_2C_0$ (1%)
$N_0C_0 > N_1C$ (1%), $N_0C_0 > N_2C$ (1%)
$N_2C > N_2C_0$ (1%).

—— oOo ——

Phosphorus:-

Significant difference -

Main N -

$N_1 > N_0$ (5%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K -

$K_0 > K$ (1%)

Main C -

$C > C_0$ (1%)

Inter NK -

$N_2K > N_0K$ (1%), $N_2K > N_1K$ (1%)
$N_1K > N_0K$ (1%), $N_0K_0 > N_0K$ (1%)
$N_2K_0 > N_0K$ (1%), $N_1K_0 > N_0K$ (1%)
$N_2K_0 > N_1K$ (1%), $N_1K_0 > N_1K$ (1%)

Inter KC - Not significant

Inter NC - Not significant

—— oOo ——

Sulphur:- /

Sulphur:-

Significant difference -

Main N
Main K
Main C
Inter NK
Inter KC
Inter NC

Not significant.

——— oOo ———

Sodium:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K -

$K_0 > K$ (1%)

Inter NK -

$N_2K > N_0K$ (1%), $N_2K > N_1K$ (1%)

$N_1K_0 > N_0K$ (1%), $N_1K_0 > N_1K$ (1%), $N_1K_0 > N_0K_0$ (1%)

$N_2K_0 > N_0K_0$ (1%), $N_2K_0 > N_1K_0$ (1%)

$N_2K_0 > N_0K$ (1%), $N_2K_0 > N_1K$ (1%), $N_2K_0 > N_2K$ (1%)

$N_2K > N_0K_0$ (1%)

Inter KC -

$K_0C > KC$ (1%), $K_0C > KC_0$ (1%)

$K_0C > K_0C_0$ (5%),

$K_0C_0 > KC_0$ (1%), $K_0C_0 > KC$ (1%)

Inter NC - Not significant.

——— oOo ———

Potassium:-/

Potassium:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%).

Main K - Not significant.

Main C -

$C > C_0$ (1%)

Inter NK -

$N_2K > N_1K$ (1%), $N_2K > N_0K$ (1%)

$N_1K > N_0K$ (1%), $N_1K > N_0K_0$ (1%)

$N_1K_0 > N_0K_0$ (1%), $N_1K_0 > N_0K$ (1%)

$N_2K_0 > N_0K_0$ (1%), $N_2K_0 > N_0K$ (1%)

$N_2K > N_0K_0$ (1%), $N_2K > N_1K_0$ (1%), $N_2K > N_2K_0$ (1%)

Inter KC -

$KC > K_0C_0$ (1%), $KC > K_0C$ (1%), $KC > KC_0$ (1%)

Inter NC - Not significant.

——— oOo ———

Manganese:-

Significant difference -

Main N -

$N_0 > N_1$ (1%), $N_0 > N_2$ (1%)

Main K - Not significant.

Main C - Not significant.

Inter NK - Not significant.

Inter KC/

Inter KC -

$KC_0 > K_0C_0$ (1%)

Inter NC -

$N_0C > N_1C$ (1%), $N_0C > N_2C$ (1%)

$N_0C > N_1C_0$ (1%), $N_0C > N_2C_0$ (1%),

$N_2C > N_1C$ (5%), $N_2C > N_2C_0$ (5%),

$N_0C_0 > N_1C_0$ (1%), $N_0C_0 > N_1C$ (1%)

$N_0C_0 > N_2C_0$ (1%), $N_0C_0 > N_2C$ (1%)

—— oOo ——

Discussion

N

The total nitrogen content of the grass increased under all the treatments except that of potassium fertiliser. The highest increase was 58.3% under the double dose of nitrogen fertiliser and the decrease was about 15% with potassium fertiliser. Even the controlled grass which received no fertiliser treatment had an increase of 50% in total nitrogen when compared with the same grass in the first cut. The increase of nitrogen in the grass was proportional to the levels of nitrogen fertiliser applied in the grass. The presence of clover tended to increase the nitrogen in the grass. The interaction of potassium fertiliser and clover improved the nitrogen of the grass. Combination of clover and nitrogen fertiliser increased the element in the grass and was proportionally better with a higher dose of nitrogen in the combination.

A single dose of nitrogen fertiliser decreased the protein nitrogen, whereas the double dose increased it. The increase was about 23% and the decrease was about 15%. The combination of nitrogen with potassium or nitrogen with clover had very little influence on the content of protein nitrogen in the plant. But the combination of potassium with clover increased the protein nitrogen in the grass.

Ca

Though nitrogen fertiliser and clover separately increased the calcium in the grass, the combination of nitrogen with clover tended to decrease the element and was proportional to the dose of nitrogen in the combination.

The/

The main action of potassium fertiliser decreased the element in the grass. The increase of calcium by the presence of clover was up to 25.5% but there was a decrease of 6.4% with a double dose of nitrogen with potassium and clover.

Mg

Clover improved the magnesium content of the grass as did the combined action of nitrogen fertiliser with clover. Potassium fertiliser tended to decrease the element in the grass.

Cl

Nitrogen fertiliser decreased the chlorine content of the grass, a double dose having more effect than a single dose. Potassium fertiliser increased the chlorine and the combined effect of potassium and clover tended to increase it. The combination of nitrogen fertiliser with clover reduced the uptake of chlorine by the grass.

P

The main action of the nitrogen fertiliser and the presence of clover was to increase the phosphorus in the grass. But potassium fertiliser acted differently and decreased the element. The combined effect of nitrogen and potassium fertilisers was a tendency to increase the phosphorus in the grass. The combinations of nitrogen or potassium fertilisers with clover had no significant effect on the uptake of the element by the grass.

S

The sulphur content of the grass decreased under all the treatments. However no significant difference could be established/

established on the main effects clover, nitrogen or potassium fertilisers. Even the interaction of nitrogen and potassium, nitrogen and clover or potassium and clover had no influence on the uptake of the element by the grass.

Na The sodium content of the grass was very small (0.05%). Nitrogen fertiliser increased it proportionally to the dose applied. In the increase of the element by the combined effect of nitrogen and potassium fertiliser, a double dose of nitrogen was more effective than a single dose. The presence of clover or the combination of nitrogen and clover had no significant role in the metabolism of the element in the grass. The action of potassium fertiliser and the interaction of clover and potassium fertiliser tended to decrease the element in the grass. The increase of the element was from 40-340% under different treatments.

K Nitrogen fertiliser and clover increased the potassium content of the grass, but potassium fertiliser alone had no significant role on the uptake of the element in the grass. The combination of nitrogen and potassium in the fertiliser increased the element in the grass, and the increment was proportional to the dose of nitrogen in the combination. The interaction of potassium and clover also favoured the uptake of the element by the grass. The increment was up to 55%.

Mn Nitrogen fertiliser decreased the manganese content of the grass. The tendency of the potassium fertiliser was to increase the element in the grass. The combination of nitrogen with clover had the same tendency of increasing the element/

element in the grass under treatments of different fertilisers. A decrease of about 40% and an increase of about 16% were obtained.

Table V(page 43) shows the behaviour of the grass under different fertilisers, when the different elements, calculated in milli equivalents, were taken into consideration. In comparison to calcium and magnesium the potassium content of the grass was very high, increasing the $\frac{K}{Ca+Mg}$ ratio beyond 2.2 The highest ratio was obtained when the grass had a double dose of nitrogen, potassium and clover. The grass receiving this treatment had also very high AA and very low EA values.

An increase in the total nitrogen in the grass of about 58.3% was obtained with nitrogen fertilisation. Even the controlled grass, which received no fertiliser, increased in nitrogen content by 50% when compared to the same grass of the first cut. Nitrogen fertiliser and clover increased the calcium in the grass, but potassium fertiliser and the combination of nitrogen and clover tended to decrease the element. The magnesium content of the grass generally increased with different treatments of fertilisers except with that of potassium which had a tendency to decrease the element in grass. Nitrogen fertiliser decreased the chlorine content of the grass and so did the combination of nitrogen with clover. Potassium fertiliser alone and in combination with clover increased the element in the grass.

Nitrogen fertiliser and clover increased the phosphorus content/

Table V

Milli equivalents per Kg of dry matter (P = 3 equivalents)

(2nd cut)

Treatment	AA Alkali Alkalinity K+Na-Cl-S	EA Alkaline Earth Alkalinity Ca+Mg-P	TA = AA+EA Total Alkalinity	VA = AA-EA Alkalinity Difference	K / Ca + Mg
Control	440.0	- 36.0	404.0	476.0	2.19
K	489.0	36.0	525.0	453.0	2.25
C	701.0	19.0	720.0	682.0	2.22
KC	565.0	9.0	574.0	574.0	2.33
N_1	949.0	31.0	980.0	918.0	2.77
N_1C	753.0	- 48.0	705.0	801.0	2.40
N_1K	661.0	-35.0	626.0	696.0	2.68
N_1KC	868.0	12.0	880.0	856.0	2.74
N_2	865.0	- 43.0	822.0	908.0	2.51
N_2C	807.0	- 31.0	776.0	838.0	2.41
N_2K	968.0	- 3.0	965.0	971.0	2.86
N_2KC	1015.0	-120.0	895.0	1135.0	3.44

content of grass but potassium fertiliser acted in the opposite direction and decreased the element in the grass. Sulphur contents decreased under all the treatments. Though the grass contained a very small amount of sodium, it was increased tremendously by the application of nitrogen fertiliser. The combination of nitrogen and potassium also brought about an increase. Though the action of potassium fertiliser and the interaction of clover and potassium had a tendency to decrease the element, other treatments increased the sodium and also reduced the wide ratio of Na:K when compared to the controlled grass. It was found that potassium fertiliser alone did not increase the potassium in the grass but in combination with nitrogen and clover it did produce an increase. The K/Ca + Mg ratio was increased beyond 2.2 by all the treatments, but the highest ratio was obtained in the grass having a double dose of nitrogen with potassium and clover. The same grass showed the highest value for AA and lowest value for EA.

Results of Third cut (23.5.57).

Table VI shows the chemical composition of the grass under different treatments with fertilisers. The next few pages show the statistical results of the main action of different treatments and the interactions of combined treatments.

Table VI

THIRD CUT (date?)

Chemical composition of grass under different treatments on dry matter basis (mean value)

(1957?)

Treatments	Total N %	Protein N %	Ash %	Silica %	Ca %	Mg %	Cl %	P %	S %	Na %	K %	Mn p.p.m.
Control	1.70	1.18	7.27	1.47	0.42	0.12	0.57	0.34	0.31	0.05	2.27	92.0
K	1.70	1.24	7.51	1.06	0.42	0.12	0.67	0.32	0.31	0.06	2.38	81.0
C	1.91	1.37	7.42	1.23	0.47	0.12	0.56	0.35	0.24	0.06	2.47	73.0
KC	1.79	1.26	7.33	1.28	0.40	0.12	0.59	0.31	0.30	0.05	2.45	85.0
N_1	2.61	1.80	7.32	0.82	0.52	0.15	0.49	0.38	0.31	0.17	2.44	102.0
N_1C	2.35	1.73	7.75	0.95	0.48	0.14	0.50	0.38	0.31	0.11	2.68	100.0
N_1K	2.39	1.70	7.99	0.96	0.38	0.14	0.55	0.37	0.31	0.05	2.99	95.0
N_1KC	2.31	1.70	8.10	0.84	0.42	0.14	0.59	0.39	0.31	0.05	3.04	112.0
N_2	2.97	2.11	7.91	0.82	0.52	0.16	0.54	0.41	0.33	0.21	2.55	94.0
N_2C	3.18	2.20	8.30	0.79	0.54	0.17	0.47	0.42	0.34	0.24	2.92	108.0
N_2K	2.97	2.14	9.33	0.74	0.44	0.16	0.49	0.40	0.33	0.07	3.42	100.0
N_2KC	3.00	2.28	9.47	0.90	0.42	0.16	0.51	0.40	0.35	0.06	3.65	106.0

Statistical Results

Total Nitrogen:-

Significant Difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K -

$K_0 > K$ (5%)

Main C - Not significant.

Inter NK - not significant.

Inter KC - Not significant.

Inter NC -

$N_1C > N_0C$ (1%), $N_2C > N_0C$ (1%), $N_2C > N_1C$ (1%)

$N_1C_0 > N_1C$ (5%)

——— oOo ———

Protein Nitrogen:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K - Not significant.

Main C -

$C > C_0$ (1%)

Inter NK - Not significant.

Inter KC - Not significant.

Inter NC -

$N_0C > N_0C_0$ (1%), $N_1C > N_0C$ (1%)

$N_2C > N_0C$ (1%), $N_2C > N_1C$ (1%)

——— oOo ———

Calcium:-

Significant difference -

Main N -

$N_1 > N_0$ (5%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%).

Main K -

$K_0 > K$ (1%)

Main C - Not significant.

Inter NK -

$N_0K_0 > N_0K$ (1%), $N_0K_0 > N_1K$ (1%), $N_2K > N_1K$ (1%)

Inter KC - Not significant.

Inter NC - Not significant.

——— oOo ———

Magnesium:-

Significant difference -

Main N -

$N_2 > N_0$ (1%)

Main K - Not significant.

Main C - Not significant.

Inter NK - Not significant.

Inter KC - Not significant.

Inter NC - Not significant.

——— oOo ———

Chlorine:-

Significant difference -

Main N/

Main N -

$N_0 > N_1$ (1%), $N_0 > N_2$ (1%), $N_1 > N_2$ (1%)

Main K -

$K > K_0$ (1%)

Main C - Not significant.

Inter NK -

$N_0K > N_1K$ (1%), $N_0K > N_2K$ (1%), $N_0K > N_0K_0$ (1%)

$N_1K > N_2K$ (1%)

Inter KC - Not significant.

Inter NC -

$N_0C > N_1C$ (5%), $N_0C > N_2C$ (1%), $N_0C_0 > N_0C$ (1%)

$N_0C_0 > N_1C$ (1%), $N_0C_0 > N_2C$ (1%), $N_0C_0 > N_1C_0$ (1%)

$N_0C_0 > N_2C_0$ (1%), $N_1C > N_2C$ (1%)

——— oOo ———

Phosphorus:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K - Not significant.

Main C - Not significant.

Inter NK - Not significant.

Inter KC - Not significant.

Inter NC - Not significant.

——— oOo ———

Sulphur-/

Sulphur:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K -

$K > K_0$

Main C - Not significant.

Inter NK -

$N_2K > N_1K$ (1%), $N_2K > N_0K$ (1%), $N_2K > N_0K_0$ (1%)

$N_2K > N_1K$ (1%), $N_0K > N_0K_0$(1%), $N_1K > N_0K_0$ (1%)

Inter KC - Not significant.

Inter NC - Not significant.

—— oOo ——

Sodium:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K -

$K_0 > K$ (1%)

Main C - Not significant.

Inter NK -

$N_1K_0 > N_1K$ (1%), $N_1K_0 > N_0K$ (1%), $N_1K_0 > N_0K_0$ (1%)

$N_2K_0 > N_2K$ (1%), $N_2K_0 > N_0K_0$ (1%), $N_2K_0 > N_1K$ (1%)

$N_2K_0 > N_0K$ (1%), $N_2K_0 > N_1K_0$ (1%).

Inter KC - Not significant.

Inter NC - /

Inter NC -

$N_1C > N_0C$ (1%), $N_2C > N_0C$ (1%)

$N_2C > N_1C$ (1%), $N_1C_0 > N_1C$ (1%)

——— oOo ———

Potassium:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%), $N_2 > N_1$ (1%)

Main K -

$K > K_0$ (1%)

Main C -

$C > C_0$ (1%)

Inter NK -

$N_1K > N_0K$ (1%), $N_2K > N_0K$ (1%), $N_2K > N_1K$ (1%)

$N_1K > N_1K_0$(1%), $N_2K > N_2K_0$(1%)

Inter KC - Not significant.

Inter NC - Not significant.

——— oOo ———

Manganese:-

Significant difference -

Main N -

$N_1 > N_0$ (1%), $N_2 > N_0$ (1%)

Main K - Not significant.

Main C - Not significant.

Inter NK - Not significant.

Inter KC - Not significant.

Inter NC - Not significant.

——— oOo ———

Discussion

The total nitrogen of the grass was increased by the application of nitrogen fertiliser and the increment was proportioned to the amount of fertiliser applied. The main action of potassium fertiliser was to effect a reduction of the nitrogen in the grass. Though clover alone increased the nitrogen content of the grass, the main action of clover was not significant. Neither the combination of nitrogen and potassium fertiliser nor that of potassium with clover had any significant effect on the uptake of the element by the grass. In the combination of nitrogen and clover, a double dose of the fertiliser increased the nitrogen whereas a single of the fertiliser tended to decrease the element in the grass. An increase of 87% was obtained by the application of a double dose of nitrogen fertiliser with clover.

The nitrogen fertiliser increased the protein nitrogen of the grass and the increment was proportioned to the dose of fertiliser applied. The presence of clover increased the element in the grass. The main action of potassium or combinations of nitrogen with potassium or of potassium with clover had no significant effect to the protein nitrogen in the grass. The combination of nitrogen and clover increased the protein nitrogen and the increment was proportional to the dose of nitrogen fertiliser in the combination. An increase of 93% of the element was recorded for the application of a double dose of nitrogen, with potassium and clover.

The/

Ca The calcium content of the grass was increased by nitrogen fertiliser and the increase was proportional to the level of fertiliser applied. But potassium fertiliser acted otherwise and tended to decrease the element in the grass. Though clover alone improved the uptake of calcium, the main action of clover was not statistically significant. The combination of potassium and clover or nitrogen and clover also had no significance in the metabolism of the element in the grass. Combination of nitrogen and potassium had a depressing effect on the uptake of calcium and a single dose of nitrogen in the combination decreased the element more than the double dose.

Mg In the case of magnesium, nitrogen fertiliser tended to increase the element in the grass proportionally to the dose of the fertiliser used. The main actions of potassium or clover were not significant. Similarly no significance could be established for the interactions of nitrogen and potassium, potassium and clover or nitrogen and clover on the uptake of the element.

Cl The chlorine content of the grass was reduced by the application of nitrogen fertiliser and the reduction was proportional to the amount of fertiliser applied. But the potassium fertiliser increased the element in the grass. The main action of clover on the chlorine content of grass was not found to be significant. The combination of nitrogen and potassium decreased the element in the grass and/

and the decrease was proportional to the dose of nitrogen fertiliser. The interaction of nitrogen and clover also acted in the same manner decreasing the chlorine content proportionally to the level of nitrogen fertiliser in the combination. On the uptake of the element by the grass, the combination of potassium and clover had no significance.

The phosphorus content of grass was increased by the application of nitrogen fertiliser and it was proportional to the dose of fertiliser. Though the presence of clover slightly increased the element and the application of potassium fertiliser slightly decreased the element, the main actions of clover or potassium were not found to be statistically significant. None of the interactions of nitrogen with potassium, potassium with clover or nitrogen with clover had any significance on the metabolism of the element in the grass.

Nitrogen fertiliser increased the sulphur content of grass proportionally to the dose applied. Though clover alone decreased the element, the main action of clover on the uptake of the element was not significant. The main action of potassium fertiliser, used as potassium sulphate, tended to increase the sulphur in the grass. The interaction of nitrogen and potassium increased the sulphur content of grass and the increase was proportional to the dose of nitrogen in the combination. The combination of nitrogen and clover or potassium and clover had no significance in the metabolism of the/

the element in the grass.

Na The grass contained very little sodium (0.05%). But nitrogen fertiliser increased the element proportionally to the dose applied. Potassium fertiliser decreased the element in the grass. The main action of clover was not significant. In the interaction of nitrogen and potassium a double dose of the fertiliser tended to increase the sodium of the grass. The combination of nitrogen and clover also increased the uptake of the element in the grass and the increase was proportional to the dose of nitrogen fertiliser in the combination. The interaction of potassium and clover had no influence on the sodium content of grass. An increment up to 380% was obtained by the application of a double dose of nitrogen in the clover. This tremendous increase under the above mentioned treatments reduced substantially the wide ratio of Na:K in the grass.

K Potassium was increased in the grass by the nitrogen fertiliser and the increment was proportional to the dose of fertiliser used. The main actions of clover and potassium also tended to increment the potassium in the grass. The interaction of nitrogen and potassium also increased the element in the grass proportionally to the level of the nitrogen fertiliser in the combination. The combination of potassium and clover or nitrogen and clover were not significant in the uptake of the element by the grass.

Mn Nitrogen fertiliser tended to increase the manganese content of grass. The action of potassium and clover or the interaction/

interaction of nitrogen and potassium, potassium and clover and nitrogen and clover had no significance on the uptake of the manganese in the grass.

Table VII (page 56) shows the behaviour of the grass under different treatments when the different elements are expressed in milli equivalents.

The $\frac{K}{Ca+Mg}$ tended to increase with the addition of nitrogen and potassium fertilisers. The highest values of AA and the lowest for EA were obtained in the grass which received a double dose of nitrogen fertiliser with potassium and in presence of clover. This is the condition said to be conducive to the occurrence of tetany.

Not true.

Nitrogen fertiliser increased the total nitrogen contents of the grass up to 87%. The tendency of the potassium fertiliser was to effect a decrease in the nitrogen in the grass. The nitrogen fertiliser also increased the protein nitrogen of the grass. The combination of clover with nitrogen fertiliser increased the protein up to 93%. Nitrogen fertiliser increased the calcium content of the grass but potassium fertiliser decreased it. The combination of nitrogen and potassium fertilisers had also a depressing effect on the uptake of the element by the grass. The same nitrogen fertiliser increased the magnesium content of the grass. Potassium fertiliser increased the chlorine content but nitrogen fertiliser decreased it. The interactions of nitrogen and clover or nitrogen and potassium was unfavourable to the uptake of the element by the grass.

Nitrogen/

Table VII

Milli equivalents per Kg of dry matter (P = 3 equivalents)

(3rd cut)

Treatment	AA Alkali Alkalinity K+Na-Cl-S	EA Alkaline Earth Alkalinity Ca+Mg-P	TA = AA+EA Total Alkalinity	VA = AA-EA Alkalinity Difference	$\frac{K}{Ca + Mg}$
Control	249.0	- 20.0	229.0	269.0	1.88
K	273.0	0.0	273.0	273.0	1.97
C	350.0	- 4.0	346.0	354.0	1.89
KC	296.0	- 1.0	295.0	297.0	2.10
N_1	367.0	14.0	381.0	353.0	1.63
N_1C	399.0	- 13.6	386.0	412.0	1.93
N_1K	439.0	- 53.0	386.0	492.0	2.51
N_1KC	440.0	- 52.0	388.0	492.0	2.39
N_2	385.0	- 5.0	380.0	390.0	1.67
N_2C	606.0	- 6.0	600.0	612.0	1.83
N_2K	361.0	- 33.0	328.0	394.0	2.57
N_2KC	598.0	- 55.0	543.0	655.0	2.89

Nitrogen fertiliser increased the phosphorus content of the grass. Nitrogen and potassium fertilisers increased the sulphur content of the grass. The sodium content of the grass was increased by nitrogen fertiliser and decreased by potassium fertiliser. A maximum increase of 380% was obtained under the double dose of nitrogen fertiliser with clover. The combination of nitrogen and clover increased the sodium of the grass proportionally to the dose of nitrogen fertiliser in the combination. Though the combination of nitrogen and potassium fertilisers decreased the sodium, when compared with grass that received only nitrogen fertiliser, a double dose of nitrogen fertiliser in the combination tended to increase the element in the grass.

Clover increased the potassium content of grass as did the potassium and nitrogen fertilisers. The interaction of nitrogen and potassium also increased the potassium in the grass. Nitrogen fertiliser increased the manganese content of grass.

The $\frac{K}{Ca+Mg}$ ratio in the grass was increased by the nitrogen and potassium fertiliser and the highest value of AA and the lowest value of EA were obtained under the same treatments, making the grass conducive to the occurrence of tetany.

GENERAL DISCUSSION

1. The total nitrogen of the grass was increased by the application of nitrogen fertiliser. The increment was found to be proportional to the amount of fertiliser used. An increment of 58.3 to 87% was obtained in different cuts. The presence of clover also increased the nitrogen. But an application of a single dose of nitrogen with clover tended to decrease the element in the grass when compared to the grass that had clover without nitrogen. The tendency of the potassium fertiliser was also to decrease the nitrogen. The control grass in the second cut had 27 to 50% more nitrogen than the third and first cuts respectively.

2. A double dressing of nitrogen fertiliser increased the protein nitrogen in the grass, but the single dressing of the fertiliser tended to decrease the protein. The presence of clover and the combination of potassium and clover also increased the protein in the grass. An increase of 23 to 97% in the protein was found in the three cuts under different treatments.

3. The effect of nitrogen fertiliser on the calcium content of grass was different for different cuts. In the first cut the fertiliser decreased the element proportionally to dose applied. In the second and third cuts it increased the element. Potassium fertiliser decreased the element. The combination of nitrogen and clover also tended to decrease the element in the grass.

4. In the first cut the magnesium content of the grass was reduced by almost all the treatments and the decrease was up to/

to 35%. In the second the presence of clover and the interaction of nitrogen and clover improved the magnesium content of the grass. This time too the potassium fertiliser decreased the magnesium. In the third cut nitrogen fertiliser tended to increase the magnesium content of grass. The magnesium content of the control grass differed in the different cuts.

5. In all the cuts nitrogen fertiliser decreased the chlorine content of grass but the potassium fertiliser increased it. The combination of potassium and clover increased the chlorine content but the combination of nitrogen and clover or nitrogen and potassium tended to decrease the element in the grass. An increase of 18 to 88% and a decrease of 15 to 18% were found under different treatments in the three cuts.

6. In the first cut nitrogen fertiliser decreased the phosphorus content of the grass whereas in the second and third cuts it increased the element in the grass. The potassium fertiliser increased the element in the first cut but in the second and third cuts it decreased the amount of phosphorus in the grass. In the first cut clover alone or its combination with nitrogen decreased the element.

7. In the first and second cuts the sulphur content of the grass was generally decreased by different treatments. In the third cut the tendency of the different treatments was to effect an increase of the element in the grass.

8./

8. The grass contained very small amounts of sodium, but almost all the treatments, except potassium, increased the sodium in the grass. The action of the latter fertiliser was to decrease the element. The increase of the element varied from 237.5 to 380% in the different cuts. This large increase narrowed down the wide ratio of Na:K when compared to the control grass.

9. Nitrogen fertiliser, clover and potassium separately increased the potassium content of the grass. Most of the combination of different treatments also increased the element. A double dressing of nitrogen with potassium and clover increased the element by 12-61% in different cuts.

10. In the first cut potassium fertiliser alone and in combination with clover tended to reduce the manganese content of grass. In the second cut nitrogen fertiliser reduced the element but potassium fertiliser and the combination of nitrogen and clover increased the element. In the third cut nitrogen fertiliser tended to reduce the element.

11. The double dressing of nitrogen fertiliser with potassium and clover increased the $\frac{K}{Ca+Mg}$ ratio in the grass. The highest value for AA and the lowest for EA were also obtained under this treatment. These are the conditions conducive to tetany as observed by the Dutch workers.

Green (1939) said that there were "grass tetany years" and years in which the incidence was almost negligible and that sometimes the disease seemed to be associated with climatic/

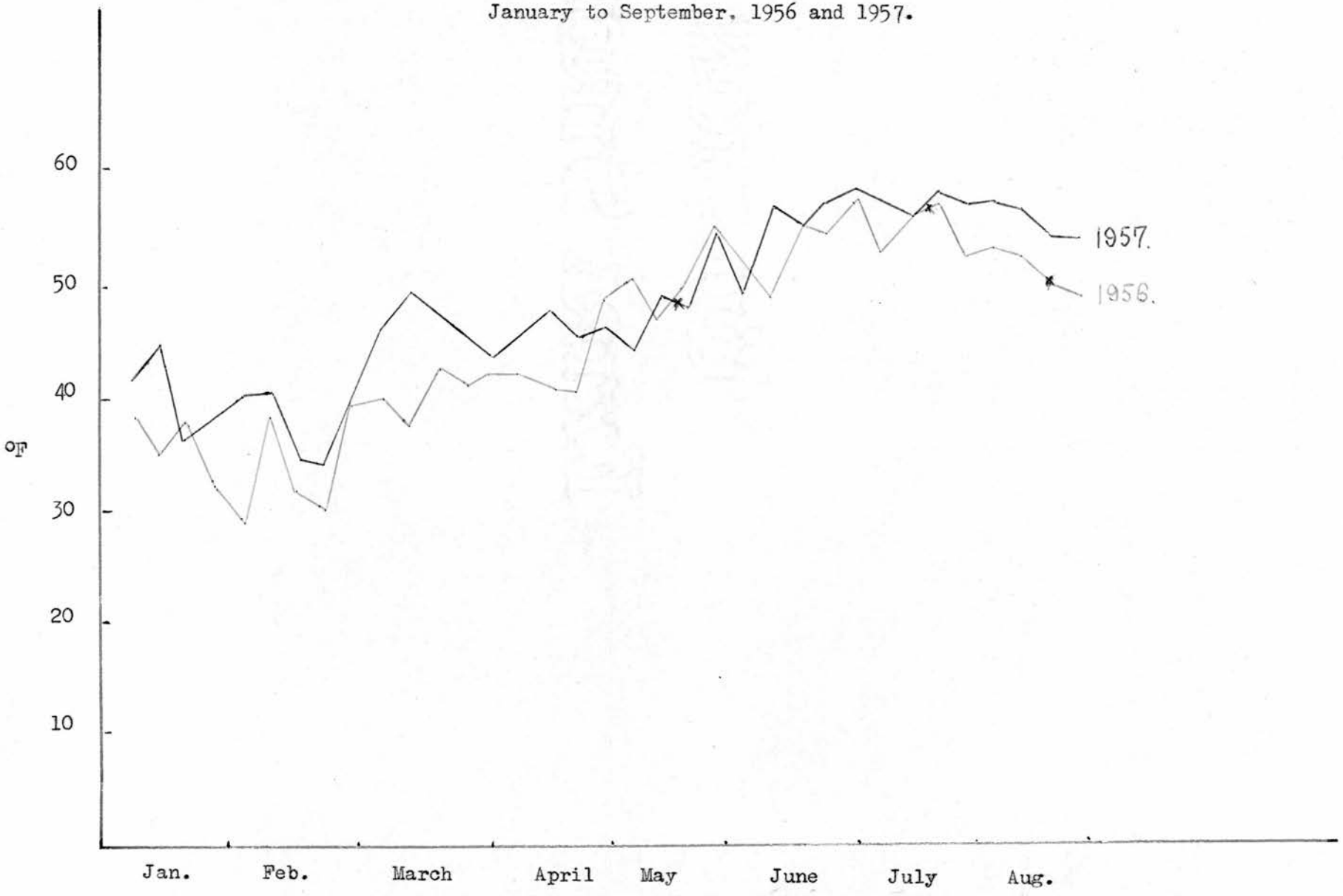
climatic conditions that encouraged a sudden flush of grass growth. Allcroft (1947) found the lowest serum magnesium levels in cold, wet and windy weather, with little sun and no growth of grass. Deys et al.(1951) have shown that there were also fluctuations in the disease depending on the season. These conditions were also thought to be possible agents conducive to the outbreak of grass tetany.

Taking into consideration the contents of calcium and magnesium in the grass, the results of the first cut in 1956 show that different treatments decreased the elements to a great extent in comparison to the other cuts. Some elements were affected, by certain treatments, differently at different seasons. This led us to study the temperature and other weather reports of the years, 1956 and 1957, the period during which the grasses were grown and received different treatments. The graph on page 62 shows the weekly mean temperature variations during two seasons. The weather reports recorded that in 1956, the weather was dull, rainy and windy up to July. From August onwards the weather changed to sunny and warm. In 1957, up to March, the weather was dull and cloudy. But from April onwards it changed to sunny, warm and bright periods.

It is obvious from the above reports and a visual inspection of the temperature graph that there were considerable differences in weather between the two seasons. It is highly probable that weather conditions can influence the uptake of minerals from the soil and it is likely that this factor may have had an important effect in the metabolism of elements in the three different cuts of grass.

Graph showing variations in mean weekly temperature

January to September, 1956 and 1957.



M I S C E L L A N E O U S S A M P L E S

(From hill, tetany and non tetany areas)

In addition to the samples analysed from fertilised plots at Bush, twenty-one different samples of grasses were also analysed for major elements. A number of the samples were obtained from suspected tetany areas. The majority of these samples were not collected by the author. A description of the samples and areas from which they were obtained is given below.

Samples 1-5. These were collected from Sourhope Hill (Roxburghshire). Sample 1 was taken in June, 1950 and Sample 2 in June, 1952, the grasses present being a mixture of Molinia caerulea, Nardus stricta and Festuca ovina. Pure cuts of these species were also analysed separately as samples 3, 4 and 5 respectively. These cuts were collected in August 1952.

Sample 6 was collected from Skeds Bush Farm (near Gifford, East Lothian) from a reseeded field which has been down for three years. This sample was taken on the 5th of March, 1956, three days after three Blackface ewes had died before lambing from suspected tetany.

Sample 7 was taken from a hill on Lower Luthrie Farm, Fife. The hill consisted of rough grazing having mainly Fescue and creeping thistles. The sample was taken on 3rd of April, 1953. The previous spring the farmer had lost four suckling cows and four ewes after lambing. The trouble was presumed to be precipitated by mild spring weather and flush of growth of grass.

Sample/

Sample 8 was collected from different parts of the above-mentioned hill between two areas of woodland. It was a good permanent pasture with a large quantity of nettles. Cattle congregated here in bad weather. The sample was collected on the 3rd of April, 1953.

Sample 9 was collected from Down Field Farm, Fife. It was first year's grass after oats. The farmer lost fifteen cattle from suspected tetany. The sample was taken on the 2nd of July, 1953.

Sample 10 was from the same area but treated with 1 cwt./acre of K_2SO_4, $MgSO_4$, $6H_2O$, and taken on the 2nd of July, 1953.

Sample 11 was taken from ley field. It was grass from a second cut at Forteviot, Bankhead Farm, Perthshire.

Sample 12 was third cut grass from the same Bankhead Farm, but the field was treated with 4 cwt./acre of nitrochalk (2 treatments). Both the samples 11 and 12 were taken on 13th July, 1953.

Samples 13-15. These were collected from a farm, MacAlistair-Hall, Braidwood, Penicuik. The farmer lost two heifers from suspected tetany in 1954. He also lost 3 ewes after lambing between 16-20th April, 1956.

Case 1. Ewe grazed in the area I (sample 13), reseeded 6th year, then put on the hill (reseeded 6th year) died after 10 days.

Case 2. Ewe grazing in the area II (sample 14) reseeded 4th year/

4th year, then put to area III (sample 15) for 2 days, then on the hill for 1 day. It was found dead next day. The pasture was predominantly perennial rye grass, with Cocksfoot, a little timothy and also white and red clover. The samples were taken on the 26th of April, 1956.

Sample 16 was collected from Craw Hill, Bathgate. The field was 7 acres of 8-year-old grass and well fertilised. Heavy nitrogen dressings were balanced with potassium. The sample was taken on the 10th April, 1956.

Sample 17 was collected from Gavieside Farm, West Calder, West Lothian. Four cases of staggers were reported on this field at the beginning of May, 1956, but only one cow died on the 16th May. The sample was taken on the 17th of May, 1956.

Sample 18 was collected from "Dairy Field", Edgehead, Pathhead. It was first year grass and was being strip grazed. Italian rye grass was by far the most abundant single species, followed by perennial rye grass. Cocksfoot was present but did not contribute largely to the produce. The clovers were relatively scarce. The most prominent was red clover but alsike and white clover were also present. In this field there was a fatal case on the 6th of May, 1956, and a suspected one of tetany on the 9th of May, out of 35 milking cows. The sample was taken on the 10th of May, 1956.

Samples 19 and 20 were collected from Bass Rock Farm, North Berwick and were fourth year grass. Perennial ryegrass was decidedly the dominant species contributing by far the

largest proportion of the herbage. The only other grass present in any quantity was cocksfoot but this was not tufty in character. White clover was present throughout the sward but more abundant on the section sown with a late strain of grass (sample 20), in which grass growth was less strong; very few weeds were present. A milking cow died of tetany on the 7th May and there was a suspected case on the 9th of May. The early strain of grass (sample 19) were being grazed at this time by about 40 cows. Samples were collected on the 18th May, 1956.

Sample 21 was a normal grass from Scrapiefield, Moredun Institute, old grass paddock. There were no cases of tetany in the sheep grazing in the field. The main part of the paddock carried a mixed grassy herbage, the most abundant constituent of which was red fescue. Associated with this the following grass species occurred: Common bent, Perennial rye grass, Sweet vernal grass, Smooth stalked meadow grass, Crested dogstail, Meadow foxtail, Cocksfoot, Yorkshire fog, and Soft brome grass. Very little clover was present.

Table VIII, page 67, shows the chemical composition of the grass collected from the different areas mentioned above and Table IX, page 68, shows the elements, calculated in milli equivalents.

Discussion:

The chemical analyses of samples 1 and 2 show how the same grass species collected from the same area in two different years differed in composition. Sample 2 had more of most of the/

Table VIII

Chemical composition of grass (collected from hill, tetany affected and normal areas) on dry matter basis

Sample No.	Total N %	Protein N %	Ash %	Silica %	Ca %	Mg %	Cl %	P %	S %	Na %	K %	Mn p.p.m.
+1	0.92	0.83	6.86	5.22	0.11	0.08	0.13	0.28	0.15	0.05	0.75	274.0
2	1.71	1.35	7.32	4.39	0.15	0.12	0.34	0.21	0.17	0.03	1.68	418.0
+3	2.22	1.64	5.99	2.70	0.24	0.19	0.37	0.19	0.33	0.03	1.78	415.0
+4	1.07	0.71	8.94	6.23	0.18	0.10	0.28	0.18	0.14	0.04	0.99	558.0
+5	1.61	1.24	6.89	3.23	0.21	0.15	0.28	0.26	0.20	0.07	1.57	436.0
-6	1.93	1.56	10.66	4.71	0.48	0.16	0.39	0.25	0.25	0.08	0.79	329.0
7	2.51	1.98	14.70	8.29	0.64	0.34	0.26	0.33	0.27	0.07	1.75	164.0
8	1.67	1.27	8.68	5.71	0.48	0.15	0.16	0.23	0.21	0.06	0.51	285.0
9	1.50	1.01	6.94	2.37	0.41	0.18	0.24	0.27	0.22	0.08	1.71	71.0
10	1.41	0.96	6.68	2.13	0.42	0.18	0.27	0.35	0.25	0.25	2.94	77. 0
11	1.74	1.27	6.61	1.30	0.46	0.18	0.33	0.33	0.25	0.07	1.55	49.0
12	4.47	3.51	10.80	1.99	0.55	0.22	0.35	0.45	0.47	0.08	2.08	80.0
-13	3.31	2.76	13.77	7.04	0.56	0.22	0.38	0.38	0.31	0.43	2.95	107.0
-14	2.42	2.01	9.35	4.43	0.60	0.17	0.33	0.32	0.25	0.08	2.09	187.0
-15	3.54	3.09	9.89	2.36	0.57	0.20	0.56	0.41	0.35	0.07	1.21	151.0
16	4.21	3.37	9.44	1.42	0.56	0.17	0.28	0.41	0.36	0.12	3.59	85.0
-17	3.65	2.91	10.47	1.70	0.55	0.22	0.40	0.50	0.37	0.21	3.05	155.0
-18	2.83	1.96	10.92	2.64	0.67	0.17	* 1.45	0.44	0.25	0.14	3.53	91.0
-19	2.95	1.98	10.89	2.80	0.67	0.19	0.39	0.48	0.33	0.09	2.40	79.0
- 20	3.50	2.39	14.33	4.34	0.72	0.21	0.29	0.55	0.42	0.75	3.06	92.0
21	2.53	2.05	7.54	1.19	0.61	0.22	0.07	0.33	0.30	0.56	1.81	142.0

* read as 0·45?

Table IX.

Milli equivalents per Kg dry matter (P = 3 equivalents)

Sample No.	AA Alkali Alkalinity K+Na-Cl-S	EA Alkaline Earth Alkalinity Ca+Mg-P	TA = AA+EA Total Alkalinity	VA = AA-EA Alkalinity Difference	K / Ca + Mg
1	84.0	- 150.0	- 66.0	234.0	1.51
2	238.0	- 29.0	209.0	267.0	2.45
3	158.0	92.0	250.0	66.0	1.65
4	104.0	- 2.0	102.0	106.0	1.47
5	228.0	- 23.0	205.0	251.0	1.76
6	- 29.0	130.0	101.0	- 159.0	0.54
7	237.0	280.0	517.0	- 43.0	0.75
8	- 20.0	141.0	121.0	- 161.0	0.36
9	267.0	92.0	359.0	175.0	1.24
10	629.0	20.0	649.0	609.0	2.10
11	177.0	59.0	236.0	118.0	1.05
12	175.0	20.0	195.0	155.0	1.17
13	641.0	92.0	733.0	549.0	1.64
14	321.0	130.0	751.0	191.0	1.22
15	- 37.0	51.0	14.0	- 88.0	0.69
16	667.0	22.0	689.0	645.0	2.19
17	516.0	- 29.0	497.0	545.0	1.70
18	399.0	48.0	447.0	351.0	1.91
19	337.0	26.0	363.0	311.0	1.25
20	765.0	0.0	765.0	765.0	1.47
21	499.0	166.0	665.0	333.0	0.96

the elements, but the increase in potassium of 124% (compared to the same species in sample 1), raised the $\frac{K}{Ca+Mg}$ ratio to a point said to be conducive to tetany. In general the hill grasses were low in mineral constituents excpet manganese which was very high, probably because of the greater degree of soil acidity.

In the areas where there were suspected cases of tetany, though some of the grasses showed highest Alkali Alkalinity values, no association with the $\frac{K}{Ca+Mg}$ could be established. One of the reasons for this might have been due to the "contamination" of the grass by clover and weeds. Clover and weeds have generally more calcium and magnesium than grass and the admixture of the grass with clover or weeds would lower the ratio (Kemp and 'T. Hart, 1957). In this connection it may be mentioned that in controlling weeds in pastures noninjurious plants should be protected from complete eradication. The ruthless use of chemical and biological means (phytohormones) over whole areas of permanent grassland can lead to the evolution of plant stands poor in species and to the occurrence of nutritional disturbances in the grazing animals (Gruenigen, 1949).

SUMMARY

Though for more than 20 years many hypotheses have been put forward to explain hypomagnesaemic tetany in sheep and cattle, the primary cause of the abnormality has not yet been established. Scotland has been experiencing serious trouble from the disease but extensive work on the problem has not been carried out.

This investigation was undertaken to examine the variations in the mineral constituents of grass under different treatments with fertilisers that are generally being practised by farmers.

Grass was grown at Bush House Experimental Farm, on 12 plots with 3 replicates of each treatment and chemically analysed. The results were also analysed statistically for the establishment of the significance of the action of different treatments, and the interactions between treatments, on the nutrients of the grass.

The results showed that the total nitrogen of the grass was increased up to 87% by nitrogen fertiliser. This large increase of nitrogen may cause trouble in the animals' assimilation of magnesium in early grazing in spring time. (Head and Rook, 1955).

An increase of 97% in protein nitrogen was also found with the application of nitrogen fertiliser.

Nitrogen fertiliser acted differently on the calcium and magnesium content of grass in different springs. In one spring it reduced the elements and in a second it tended to increase/

increase the elements. In the second cut both elements were increased in the grass by the fertiliser. But the tendency of the potassium fertiliser was always to reduce the calcium and magnesium in the grass.

In all three cuts nitrogen fertiliser reduced the chlorine content of grass whereas the potassium fertiliser increased it. The combination of potassium and clover also increased this element but the combinations of nitrogen and clover or nitrogen and potassium tended to reduce it. A deficiency of chlorine in grass may be responsible for the convulsions in hypomagnesaemic tetany (Green Berg and Cuthbertson, 1942).

Nitrogen fertiliser reduced the phosphorus in grass in the first cut but in second and third cuts it increased the element. The potassium fertiliser also acted differently in different seasons.

The sulphur content of grass was decreased by different treatments in the first and second cuts. In the third cut the element tended to increase under different treatments.

Most of the treatments increased the sodium content of grass except potassium fertiliser which tended to reduce the element. An increase of 237 to 380% in the element was obtained in different cuts of grasses. This large increase narrowed the wide ratio of Na:K when compared with that of the control grass.

The potassium content of the grass was increased by the different treatments with fertiliser. An increase of 61% in/

in the element was found.

The manganese content of the grass varied differently in different seasons and with different fertilisers.

A higher dressing of nitrogen fertiliser with potassium increased the $\frac{K}{Ca+Mg}$ ratio in the grass beyond 2.2. Higher values for Alkali Alkalinity and lower values for Alkaline Earth Alkalinity were also found under the above mentioned treatment. These were the conditions observed by Dutch workers that make the grass conducive to tetany.

Analysis of the grass from suspected tetany and non-tetany areas revealed that hill grasses have generally low mineral contents with the exception of manganese which is very high. Though higher values for Alkali Alkalinity were noted in some grasses from suspected tetany areas, a relationship between the $\frac{K}{Ca+Mg}$ ratio and the tetany and non-tetany grasses could not be established. This might have been due to admixture of clover and weeds with grass. The weeds and clover have larger calcium and magnesium contents than grass and would thus lower down the ratio.

F I R S T C U T

Chemical composition of the grass under different treatments on dry matter basis

	Control				K				C				KC			
Constituents	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
Total N	1.43	1.45	1.44	1.44	1.55	1.52	1.48	1.52	1.87	1.85	1.77	1.83	2.02	1.96	1.98	1.99
Protein N	1.30	1.28	1.35	1.31	1.32	1.30	1.15	1.28	1.40	1.40	1.36	1.39	1.84	1.78	1.65	1.76
Ash	12.78	11.34	10.46	11.53	11.16*	9.48	9.13	9.31	10.47	13.33*	9.63	10.05	10.51	9.39	13.42*	9.95
Silica	4.099*	2.99	2.61	2.80	2.55	2.55	2.42	2.51	2.32	5.11*	2.46	2.39	2.10	1.82	5.92*	1.96
Ca	0.55	0.55	0.55	0.55	0.48	0.51	0.50	0.50	0.54	0.59	0.54	0.56	0.46	0.47	0.48	0.47
Mg	0.24	0.23	0.22	0.23	0.14	0.16	0.16	0.15	0.19	0.19	0.18	0.19	0.16	0.14	0.16	0.15
Cl	0.42	0.42	0.40	0.41	0.75	0.76	0.79	0.77	0.51	0.55	0.51	0.52	0.42	0.38	0.43	0.41
P	0.42	0.39	0.39	0.40	0.42	0.42	0.40	0.41	0.32	0.30	0.27	0.30	0.39	0.39	0.33	0.37
S	0.39	0.46	0.44	0.43	0.30	0.28	0.26	0.28	0.35	0.37	0.33	0.35	0.31	0.30	0.31	0.31
Na	0.07	0.08	0.10	0.08	0.08	0.06	0.09	0.08	0.09	0.07	0.09	0.08	0.15	0.13	0.12	0.13
K	3.24	3.38	3.46	3.36	2.66	2.62	2.72	2.67	3.37	3.38	3.34	3.36	3.91	3.62	3.82	3.78
Mn (p.p.m.)	102.0	143.0	880	111.0	113.0	162.0	130.0	135.0	79.0	193.0	151.0	141.0	95.0	105.0	126.0	109.0

* These figures were not included in the mean.

Chemical composition of grass under different treatment on dry matter basis

	N_1				N_1C				N_1K				N_1KC			
Constituents	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
%																
Total N	1.69	1.51	1.60	1.60	1.92	1.72	1.71	1.78	1.97	1.93	1.79	1.90	1.81	1.81	1.77	1.80
Protein N	1.41	1.26	1.29	1.32	1.30	1.37	1.24	1.30	1.31	1.69	1.46	1.49	1.22	1.20	1.36	1.26
Ash	8.93	8.76	8.01	8.57	9.99	10.20	9.71	9.97	9.52	8.95	8.64	9.04	9.53	10.56	9.10	9.73
Silica	1.53	1.43	1.86	1.61	2.17	2.90	2.85	2.67	1.58	2.30	1.78	1.89	2.68	2.76	2.26	2.57
Ca	0.55	0.54	0.50	0.53	0.47	0.56	0.49	0.51	0.44	0.48	0.45	0.46	0.46	0.48	0.48	0.47
Mg	0.17	0.17	0.17	0.17	0.19	0.17	0.18	0.18	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.17
Cl	0.39	0.36	0.36	0.37	0.36	0.37	0.39	0.37	0.44	0.44	0.45	0.44	0.42	0.42	0.40	0.41
P	0.31	0.32	0.28	0.30	0.36	0.33	0.39	0.36	0.36	0.38	0.38	0.37	0.33	0.33	0.30	0.32
S	0.29	0.29	0.28	0.29	0.24	0.30	0.25	0.26	0.32	0.35	0.34	0.34	0.28	0.29	0.29	0.29
Na	0.14	0.12	0.13	0.13	0.12	0.12	0.14	0.13	0.12	0.14	0.12	0.13	0.09	0.07	0.09	0.08
K	3.00	2.62	2.53	2.72	3.04	2.95	2.89	2.99	3.41	2.79	2.78	2.99	3.38	3.09	3.23	3.23
Mn (p.p.m.)	180.0	184.0	132.0	165.0	133.0	170.0	127.0	143.0	102.0	129.0	125.0	119.0	82.0	112.0	136.0	110.0

Chemical composition of grass under different treatments on dry matter basis

Constituents	N_2				N_2C				N_2K				N_2KC			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
%																
Total N	2.33	2.32	2.27	2.31	2.04	2.25	2.01	2.10	2.35	2.28	2.02	2.22	1.91	1.97	1.93	1.94
Protein N	1.82	1.70	1.48	1.67	1.53	1.64	1.73	1.63	1.76	1.83	1.70	1.76	1.29	1.30	1.27	1.29
Ash	10.73	8.71	8.69	9.38	9.66	10.00	9.00	9.55	11.21	10.56	9.44	10.40	9.74	9.52	10.26	9.84
Silica	2.52	2.12	1.99	2.21	1.76	1.47	1.86	1.70	2.37	2.66	2.72	2.58	1.96	1.58	2.08	1.87
Ca	0.49	0.43	0.49	0.47	0.50	0.53	0.54	0.52	0.49	0.46	0.44	0.46	0.43	0.44	0.45	0.44
Mg	0.18	0.16	0.18	0.17	0.17	0.19	0.19	0.18	0.18	0.17	0.18	0.18	0.16	0.16	0.17	0.16
Cl	0.37	0.30	0.38	0.35	0.37	0.41	0.41	0.40	0.46	0.51	0.48	0.48	0.51	0.54	0.55	0.53
P	0.33	0.34	0.32	0.33	0.36	0.37	0.35	0.36	0.40	0.31	0.33	0.35	0.36	0.37	0.35	0.36
S	0.42	0.40	0.41	0.41	0.29	0.30	0.31	0.30	0.29	0.31	0.30	0.30	0.34	0.32	0.37	0.34
Na	0.17	0.16	0.17	0.17	0.26	0.27	0.28	0.27	0.21	0.19	0.20	0.20	0.13	0.12	0.16	0.14
K	3.25	2.92	3.56	3.24	2.92	3.24	2.81	2.99	3.75	3.79	3.73	3.76	3.31	3.41	3.78	3.50
Mn (p.p.m.)	125.0	125.0	138.0	129.0	103.0	143.0	133.0	126.0	86.0	110.0	62.0	86.0	104.0	102.0	128.0	111.0

S E C O N D C U T

Chemical composition of grass under different treatments on dry matter basis

	Control				K				C				KC			
Constituents	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
%																
Total N	2.10	2.21	2.18	2.16	1.96	1.86	1.69	1.84	2.86	2.87	2.86	2.86	2.75	2.38	2.79	2.64
Protein N	1.39	1.73	1.57	1.56	1.43	1.43	1.46	1.44	1.55	1.39	1.47	1.47	1.89	1.37	1.56	1.61
Ash	11.72	10.79	11.25	11.25	10.21	10.34	10.43	10.33	11.66	11.75	11.69	11.70	11.78	10.43	10.99	11.07
Silica	3.24	2.46	2.61	2.77	1.82	1.73	1.92	1.83	1.89	2.00	1.98	1.96	1.83	2.10	1.98	1.97
Ca	0.47	0.46	0.47	0.47	0.46	0.46	0.46	0.46	0.59	0.59	0.60	0.59	0.49	0.49	0.49	0.49
Mg	0.21	0.20	0.20	0.20	0.20	0.19	0.20	0.20	0.23	0.23	0.23	0.23	0.22	0.20	0.24	0.22
Cl	0.45	0.46	0.46	0.46	0.55	0.61	0.58	0.58	0.48	0.46	0.50	0.48	0.62	0.60	0.62	0.62
P	0.45	0.46	0.45	0.45	0.39	0.37	0.35	0.37	0.47	0.49	0.48	0.48	0.43	0.42	0.43	0.43
S	0.55	0.50	0.52	0.52	0.40	0.40	0.42	0.41	0.41	0.45	0.43	0.43	0.47	0.43	0.43	0.44
Na	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.08	0.07	0.07	0.05	0.05	0.05	0.05
K	3.28	3.60	3.34	3.41	3.52	3.03	3.85	3.47	4.24	4.14	4.21	4.20	4.00	3.72	3.91	3.88
Mn (p.p.m.)	102.0	166.0	148.0	139.0	158.0	151.0	173.0	161.0	110.0	159.0	146.0	138.0	118.0	166.0	133.0	139.0

Chemical composition of grass under different treatments in dry matter basis

	N_1				N_1C				N_1K				N_1KC			
Constituents	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
%																
Total N	2.87	2.78	2.90	2.85	2.99	2.99	2.97	2.98	2.74	2.79	2.80	2.78	2.85	2.81	2.79	2.82
Protein N	1.36	1.44	1.30	1.37	1.48	1.31	1.37	1.39	1.39	1.36	1.25	1.33	1.47	1.49	1.30	1.42
Ash	11.49	11.22	11.39	11.37	11.68	11.44	11.46	11.53	11.29	10.47	11.03	10.93	12.59	11.29	11.75	11.89
Silica	1.62	1.62	1.63	1.62	1.59	1.66	1.61	1.62	1.66	1.27	1.58	1.50	1.26	1.67	1.47	1.47
Ca	0.55	0.54	0.57	0.55	0.53	0.52	0.53	0.53	0.45	0.44	0.45	0.45	0.50	0.50	0.49	0.50
Mg	0.21	0.21	0.21	0.21	0.22	0.23	0.21	0.22	0.20	0.18	0.19	0.19	0.26	0.23	0.24	0.24
Cl	0.36	0.39	0.38	0.38	0.48	0.47	0.49	0.48	0.54	0.54	0.55	0.54	0.45	0.47	0.44	0.45
P	0.44	0.43	0.43	0.43	0.53	0.53	0.53	0.53	0.46	0.39	0.43	0.43	0.49	0.45	0.42	0.45
S	0.42	0.40	0.35	0.39	0.46	0.47	0.44	0.46	0.41	0.38	0.36	0.38	0.39	0.37	0.43	0.40
Na	0.13	0.14	0.14	0.14	0.13	0.14	0.13	0.13	0.07	0.06	0.07	0.07	0.07	0.06	0.05	0.06
K	4.89	4.84	4.80	4.84	4.31	4.41	4.39	4.37	4.28	3.61	3.98	3.99	4.98	4.65	4.70	4.78
Mn (p.p.m.)	83.0	117.0	105.0	102.0	84.0	100.0	96.0	93.0	94.0	95.0	115.0	101.0	78.0	116.0	102.0	99.0

Chemical composition of grass under different treatments on dry matter basis

	N_2				N_2C				N_2K				N_2KC			
Constituents	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
%																
Total N	3.44	3.39	3.42	3.42	3.22	3.02	3.21	3.15	2.71	3.02	3.11	2.95	3.35	3.10	3.24	3.23
Protein N	1.95	1.72	1.88	1.85	1.45	1.57	1.54	1.52	1.59	1.66	1.60	1.62	2.15	1.69	1.92	1.92
Ash	11.14	11.35	11.28	11.26	11.96	10.87	10.99	11.01	10.22	12.00	11.57	11.26	12.94	12.38	12.73	12.68
Silica	1.28	1.57	1.37	1.41	1.65	1.53	1.75	1.64	1.78	1.42	1.67	1.63	1.43	1.53	1.64	1.53
Ca	0.54	0.53	0.55	0.54	0.51	0.49	0.54	0.51	0.53	0.54	0.54	0.54	0.45	0.41	0.47	0.44
Mg	0.22	0.22	0.23	0.22	0.23	0.22	0.23	0.23	0.21	0.19	0.23	0.21	0.22	0.21	0.21	0.21
Cl	0.33	0.35	0.34	0.34	0.43	0.42	0.43	0.43	0.37	0.34	0.29	0.37	0.41	0.39	0.46	0.45
P	0.51	0.50	0.51	0.51	0.53	0.47	0.47	0.49	0.44	0.48	0.46	0.46	0.58	0.50	0.51	0.53
S	0.40	0.40	0.40	0.40	0.36	0.40	0.38	0.38	0.42	0.40	0.42	0.41	0.43	0.44	0.43	0.43
Na	0.20	0.18	0.19	0.19	0.22	0.23	0.22	0.22	0.18	0.12	0.15	0.15	0.13	0.12	0.13	0.13
K	4.43	4.40	4.41	4.41	4.30	4.06	4.17	4.18	5.18	5.17	4.57	4.94	5.23	5.35	5.30	5.29
Mn (p.p.m.)	78.0	87.0	84.0	83.0	116.0	138.0	126.0	127.0	106.0	130.0	111.0	116.0	82.0	128.0	116.0	109.0

THIRD CUT

Chemical composition of grass under different treatments on dry matter basis

Constituents	Control				K				C				KC			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
%																
Total N	1.68	1.72	1.70	1.70	1.78	1.62	1.69	1.70	1.89	1.92	1.91	1.91	1.81	1.80	1.75	1.79
Protein N	1.28	1.16	1.10	1.18	1.35	1.09	1.27	1.24	1.44	1.31	1.37	1.37	1.30	1.26	1.22	1.26
Ash	6.99	7.52	7.31	7.27	7.20	7.88	7.45	7.51	7.55	7.32	7.38	7.42	7.25	7.28	7.47	7.33
Silica	1.45	1.35	1.60	1.47	1.03	1.08	1.07	1.06	1.34	1.14	1.20	1.23	1.14	1.25	1.45	1.28
Ca	0.42	0.41	0.43	0.42	0.41	0.43	0.42	0.42	0.46	0.48	0.48	0.47	0.39	0.40	0.40	0.40
Mg	0.12	0.12	0.13	0.12	0.11	0.13	0.13	0.12	0.12	0.12	0.12	0.12	0.11	0.12	0.13	0.12
Cl	0.57	0.59	0.54	0.57	0.67	0.66	0.68	0.67	0.54	0.57	0.56	0.56	0.56	0.60	0.62	0.59
P	0.33	0.34	0.34	0.34	0.31	0.31	0.35	0.32	0.34	0.35	0.35	0.35	0.32	0.31	0.31	0.31
S	0.28	0.32	0.32	0.31	0.31	0.30	0.32	0.31	0.25	0.23	0.24	0.24	0.28	0.30	0.33	0.30
Na	0.05	0.04	0.05	0.05	0.07	0.05	0.06	0.06	0.07	0.05	0.05	0.06	0.04	0.05	0.05	0.05
K	2.16	2.50	2.15	2.27	2.40	2.36	2.38	2.38	2.47	2.48	2.46	2.47	2.62	2.41	2.33	2.45
Mn (p.p.m.)	71.0	105.0	100.0	92.0	67.0	81.0	75.0	81.0	73.0	75.0	72.0	73.0	81.0	86.0	88.0	85.0

Chemical composition of grass under different treatments on dry matter

	N_1				N_1C				N_1K				N_1KC			
Constituents	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
%																
Total N	2.41	2.52	2.90	2.61	2.27	2.51	2.27	2.35	2.37	2.21	2.59	2.39	2.37	2.28	2.29	2.31
Protein N	1.78	1.80	1.83	1.80	1.75	1.74	1.71	1.73	1.87	1.50	1.72	1.70	1.85	1.61	1.65	1.70
Ash	7.20	7.45	7.30	7.32	7.66	8.00	7.58	7.75	7.77	7.77	8.43	7.99	8.29	8.00	8.01	8.10
Silica	0.84	0.79	0.82	0.82	1.01	0.92	0.93	0.95	0.95	0.95	0.97	0.96	0.87	0.81	0.84	0.84
Ca	0.53	0.50	0.52	0.52	0.48	0.48	0.48	0.48	0.38	0.37	0.38	0.38	0.40	0.40	0.45	0.42
Mg	0.14	0.16	0.16	0.15	0.14	0.14	0.14	0.14	0.13	0.15	0.15	0.14	0.14	0.15	0.14	0.14
Cl	0.46	0.59	0.43	0.49	0.52	0.49	0.50	0.50	0.57	0.51	0.57	0.55	0.56	0.56	0.64	0.59
P	0.34	0.37	0.43	0.38	0.36	0.39	0.38	0.38	0.37	0.36	0.37	0.37	0.37	0.38	0.42	0.39
S	0.33	0.30	0.29	0.31	0.29	0.32	0.31	0.31	0.31	0.31	0.32	0.31	0.30	0.32	0.32	0.31
Na	0.18	0.15	0.17	0.17	0.12	0.11	0.11	0.11	0.05	0.05	0.06	0.05	0.06	0.04	0.05	0.05
K	2.32	2.51	2.48	2.44	2.58	2.86	2.60	2.68	2.94	2.87	3.16	2.99	2.91	3.00	3.22	3.04
Mn (p.p.m.)	123.0	80.0	104.0	102.0	85.0	118.0	97.0	100.0	85.0	116.0	84.0	95.0	80.0	108.0	148.0	112.0

Chemical composition of grass under different treatments on dry matter basis

	N_2				N_2C				N_2K				N_2KC			
Constituents	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
%																
Total N	2.99	3.04	2.88	2.97	3.25	3.12	3.18	3.18	3.03	2.99	2.89	2.97	3.12	2.92	2.96	3.00
Protein N	2.18	2.04	2.11	2.11	2.19	2.22	2.20	2.20	2.37	2.04	2.01	2.14	2.34	2.20	2.29	2.28
Ash	8.21	8.06	7.45	7.91	8.05	8.52	8.34	8.30	9.54	9.18	9.27	9.33	9.77	9.21	9.44	9.47
Silica	0.81	0.89	0.76	0.82	0.86	0.73	0.78	0.79	0.67	0.70	0.85	0.74	0.90	0.90	0.90	0.90
Ca	0.51	0.53	0.51	0.52	0.53	0.54	0.54	0.54	0.44	0.43	0.44	0.44	0.44	0.41	0.42	0.42
Mg	0.15	0.17	0.16	0.16	0.18	0.17	0.17	0.17	0.16	0.16	0.17	0.16	0.17	0.15	0.16	0.16
Cl	0.54	0.53	0.54	0.54	0.49	0.46	0.47	0.47	0.48	0.52	0.48	0.49	0.51	0.51	0.51	0.51
P	0.45	0.39	0.38	0.41	0.40	0.43	0.43	0.42	0.40	0.40	0.39	0.40	0.43	0.39	0.38	0.40
S	0.32	0.34	0.33	0.33	0.33	0.34	0.34	0.34	0.33	0.32	0.33	0.33	0.36	0.34	0.34	0.35
Na	0.18	0.25	0.20	0.21	0.25	0.21	0.27	0.24	0.07	0.07	0.07	0.07	0.07	0.06	0.05	0.06
K	2.82	2.48	2.35	2.55	2.82	3.19	2.75	2.92	3.31	3.60	3.35	3.42	3.90	3.68	3.38	3.65
Mn (p.p.m.)	85.0	93.0	104.0	94.0	101.0	113.0	110.0	108.0	81.0	96.0	123.0	100.0	97.0	114.0	108.0	106.0

METHOD OF STATISTICAL CALCULATION

Phosphorus content of grass under different treatments in the first cut.

1a		1b		2a		2b		3a		3b	
C	0.32	0	0.42	0	0.39	C	0.30	0	0.39	C	0.27
K	0.42	KC	0.39	KC	0.39	K	0.42	KC	0.33	K	0.40
N_1	0.31	N_1C	0.36	N_1C	0.33	N_1	0.32	N_1	0.28	N_1C	0.39
N_1KC	0.33	N_1K	0.36	N_1K	0.38	N_1KC	0.33	N_1KC	0.30	N_1K	0.38
N_2	0.33	N_2C	0.36	N_2	0.34	N_2C	0.37	N_2C	0.35	N_2	0.32
N_2KC	0.36	N_2K	0.40	N_2KC	0.37	N_2K	0.31	N_2K	0.33	N_2KC	0.35

	N_0	N_1	N_2		
Without K	2.09	1.99	2.07	=	6.15
With K	2.35	2.08	2.12	=	6.55
	4.44	4.07	4.19		

	N_0	N_1	N_2		
Without C	2.44	2.03	2.03	=	6.50
With C	2.00	2.04	2.16	=	6.20
	4.44	4.07	4.19		

	Without K	With K		
Without C	3.10	3.40	=	6.50
With C	3.05	3.15	=	6.20
	6.15	6.55		

Factor/

Factor	Degree of Freedom	Sum of Squares	Variance	Factor	Significance
Total	35	0.0552			
Sub blocks	5	0.0104			
Main N	2	0.0060	0.003	5.7	5%
Main K	1	0.0045	-0.0045	8.6	1%
Main C	1	0.0025	-0.0025	4.8	5%
Inter NK	2	0.0020	-0.0010	1.9	Not Significant
Inter KC	1	0.0011	0.0011	2.1	Not Significant
Inter NC	2	0.0177	-0.0089	17.0	1%
Error	21*	0.011	-0.0005238		

* Error was confounded with the triple interaction N K C. The degrees of freedom allocated to this triple interaction (2) have therefore been added to the 19 degrees of freedom for error giving a total of 21.

Standard Error -

Main N - $\sqrt{0.0005238 \times 12} = 0.07929$

Main K and Main C - $\sqrt{0.0005238 \times 18} = 0.09712$

Inter NC - $\sqrt{0.0005238 \times 6} = 0.05694$

Significant difference -

Main N - (P = 0.05) = 0.23

(P = 0.01) = 0.29

Main/

Main K and Main C: (P = 0.05) = 0.29

(P = 0.01) = 0.35

Inter NC: (P = 0.05) = 0.17

(P = 0.01) = 0.20

Main N -

$N_0 > N_1$ (1%), $N_0 > N_2$ (5%)

Main K -

$K > K_0$ (1%)

Main C -

$C_0 > C$ (5%)

Inter NK - Not significant

Inter KC - Not significant

Inter NC -

$N_0C_0 > N_1C_0$ (1%), $N_0C_0 > N_2C_0$ (1%), $N_0C_0 > N_0C$ (1%)

$N_0C_0 > N_1C$ (1%), $N_0C_0 > N_2C$ (1%)

METHODS OF ANALYSIS

Residual Moisture, Ash and Silica

The residual moisture was determined by the procedure laid down in the Regulations of the Fertiliser and Feeding Stuffs Act of 1926, the ash content by the method proposed by Peach and Tracey (1956) and the silica content according to Piper (1942).

Moisture:

A suitable quantity of the material, usually 2 grams was weighed in a tared flat-bottomed vitreosil dish which had been ignited in the furnace for a few minutes and cooled in a desiccator. The dish and its contents were now dried in an oven maintained at 105°C for an arbitrary period of 24 hours, cooled in a desiccator and reweighed.

Ash:

A suitable quantity of grass, usually 5 grams, was weighed into a tared 75-ml. vitreosil silica basin and ignited in a muffle furnace. The muffle was kept at about 300°C until all the carbon had ceased to glow and then the temperature was raised to 450-500°C. The temperature was never allowed to rise beyond 500°C. Oxidation of the last traces of organic material was accomplished by removing the dish from the muffle allowing it to cool and adding 1-2 ml. of concentrated HNO_3, evaporating to dryness and replacing the dish in the muffle for a further hour or so. The basin with the ash was cooled and reweighed.

Silica: /

Silica:

The ash from the previous determination was carefully moistened with a little water. The basin was covered with a clockglass and 40 ml. of dilute HCl(1+1)added, pouring the acid into the covered basin so as to avoid any loss by effervescence. The basin, still covered, was placed on a water bath and digested for 20-30 minutes. The cover was removed and rinsed, 1 ml. of HNO_3 was added to oxidise any ferrous salt and the contents were evaporated to dryness. The heating was continued for half an hour to one hour to dehydrate the silica.

The dried salt was moistened with 10 ml. of dilute HCl (1+1) and a further 50 ml. of water and warmed on the water bath until all soluble salts were in solution. The solution was then filtered through a Whatman filter paper No. 42, 11 cm. Filter paper with residue and dish were washed with very hot water till the washings were neutral. The filter paper with the residue was transferred into a tared basin, dried in an oven and completely ignited in a muffle at about 600°C, cooled in a desiccator and reweighed. The filtrate was made up to a volume of 250 ml. and used for the estimation of Ca and Mg.

Calcium:

The methods recommended in Official Methods of Analysis (1955) by A.O.A.C. were used for the determination of Ca and Mg.

Procedure/

Procedure:-

An aliquot sample, generally 100 ml. of the acid extract from the previous estimation was transferred to a 250 ml. beaker and if necessary the volume was made up to 200 ml. with water, heated to boiling and 10 ml. of saturated $(NH_4)_2C_2O_4$ and a drop of methyl red were added. The solution was almost neutralised with NH_4OH and boiled until the precipitate was coarsely granular. When cool, NH_4OH was added until the colour was faint pink (pH 5.0) and allowed to stand at least 4 hours. It was filtered and the precipitate washed with water at room temperature until the filtrate was free from oxalate. The precipitate was washed into the beaker in which calcium was precipitated, first with hot water then with about 10 ml. H_2SO_4 (1 + 4) and finally with hot water. The volume was made up to about 100 ml. with water, boiled to about 70°C and titrated with $N/_{20}$ $NKMnO_4$. Finally the filter paper was added to the solution and the titration completed.

1 ml. of $N/_{20}$ $KMnO_4$ = 0.001 g. Ca.

Magnesium:

Procedure:-

The filtrate from the calcium determination was treated with 30 ml. of HNO_3 and evaporated to dryness to decompose the ammonium salts. The residue was taken up with 5 ml. of HCl and made up to 100 ml. with water; 5 ml. of 10% Na-citrate and 10 ml. of 10% $(NH_4)_2HPO_4$ solution or enough to precipitate/

precipitate all the magnesium and NH_4OH (1 + 4) were added with constant stirring (using a policeman) until solution was faintly alkaline and the precipitate formed, then 25 ml. of NH_4OH were added. The solution was stirred vigorously until precipitate was granular and allowed to stand in cool place overnight. The precipitate was filtered and washed with NH_4OH (1 + 10) till free from chloride. The precipitate was ignited and weighed as $Mg_2P_2O_7$, corrected for $MN_2P_2O_7$.

The factor for converting $Mg_2P_2O_7$ to Mg is 0.2184.

Phosphorus:

The method was based upon that of Richards and Godden and recommended by the Imperial Bureau of Animal Nutrition (1924).

Special Reagents:-

Dissolve 372 g. of powdered ammonium molybdate in about 1,500 ml. of tepid water, cool and add 25 ml. of concentrated NH_4OH and dilute to 2.5 litres.

Nitro-Ammonium Molybdate Reagent:-

Immediately before use mix 2 parts of ammonium molybdate reagent with 1 part of H_2O and 1 part of HNO_3.

Procedure:-

A 5 g. portion of the material was weighed into a 75 ml. vitreosil basin and 10 ml. of 20% calcium-acetate were added. The mixture was dried and then gently ashed to a white ash at a temperature of 500°C. The ash was digested with 10% HNO_3 on a water bath for about half an hour and filtered into

a/

a 250 ml. graduated flask. The basin and the residue into the filter paper were washed with hot 10% HNO_3 and at least 4 times with very hot water. The filtrate was allowed to cool and made to volume with water and well shaken. A 100 ml. portion was then pipetted into a 250 ml. beaker. It was made just alkaline to litmus with concentrated NH_4OH and then just acid with strong HNO_3; 20 ml. of 50% solution of ammonium nitrate were now added, the mixture heated to about 55-60°C and 25 ml. of freshly prepared ammonium-nitro-molybdate were added. After stirring vigorously with a glass rod, the precipitate was allowed to settle out overnight. It was then filtered through a Gooch crucible having a pad prepared from a mixture of asbestos in water, the beaker and precipitate being washed about 6 times with 1% $NaNH_3$ solution. The asbestos with the precipitate are transferred back to the original beaker and the crucible washed down with cold water and the washings added to the main solution. The precipitate was dissolved in a known volume of N/10 NaOH solution, about 1-2 ml. in excess being used, using 3-4 drops of phenolphthalein as indicator. The excess of NaOH was titrated by N/10 H_2SO_4. The volume of alkali used, less the volume of acid gives the volume of alkali equivalent to the phosphorus in the aliquot of the material.

1 ml. of N/10 NaOH = 0.00134 g. P.

Chlorine:

The procedure of Husband and Godden in conjunction with the modification of Caldwell and Moyer was found to be rapid and/

and reliable.

Reagent:-

Iron Alum - To 100 ml. saturated solution of $FeNH_4(SO_4)_2$, $12H_2O$, 5 ml. of concentrated HNO_3 are added.

Procedure:-

A suitable quantity of material - usually 2-5 g. was weighed into a 75 ml. vitreosil basin and mixed to a paste with approximately one quarter its weight of CaO and a little water. The mixture was dried on a water bath and then ashed at a temperature of 450-500°C. The ash was moistened with water and digested with approximately 2N HNO_3 for about an hour on water bath, filtered and washed into a 250 ml. graduated flask. One 100 ml. aliquot sample was taken and a measured quantity of excess N/20 $AgNO_3$ solution added. One ml. of nitrobenzene, 5 ml. of iron alum and 3 ml. of HNO_3 were added and the excess $AgNO_3$ was titrated against N/20 KCNS to a very pale reddish brown colour.

1 ml. N/20 $AgNO_3$ = 0.001773 g. Cl.

Sulphur:

The method is that of Martson (1938).

About 1-2 grams of the powdered material was compressed into a hard tablet by means of a die. The tablet was weighed in a tared small silica basin. The basin with the tablet was set up in the bomb and the fuse platinum wire was fixed in position. The wire was connected with the tablet by means of a piece of cotton thread. About 10 ml. of water were put in/

in the bomb. It was then assembled and screwed down tightly. This was then connected to an oxygen cylinder, oxygen was introduced to a pressure to 20 atmospheres, the valve was closed and the bomb was immersed in water in a calorimeter to detect any leakage of oxygen. The bomb was fired electrically and left to cool for about half an hour to allow acid mists to settle. When cool, the valve was opened carefully to relieve the pressure at a slow rate.

The contents of the bomb were transferred to a beaker by several washings with about 200 ml. of water. Care was taken that all the inner surface of the bomb was washed out.

The washings were acidified with 10 ml. of concentrated HCl and evaporated to dryness to remove HNO_3 and dehydrate the silica. The evaporation was repeated, using a further 5 ml. of HCl and 15 ml. of water. When the silica had been dehydrated, the residue was dissolved in 3-5 ml. of HCl and 15 ml. of warm water. It was filtered through 11 cm. Whatman No. 42 filter paper and washed completely with hot water.

The acidity of the filtrate was adjusted to approximately 0.02N by just neutralising with dilute ammonia, using methyl red as indicator, then adding 1 ml. HCl (1+1) and diluting to about 250 ml. The solution was then heated to incipient boiling and an excess of $BaCl_2$ (about 10 ml. of 10% $BaCl_2$ $2H_2O$) was added. The beaker was covered with a watch glass and maintained at a temperature of about 100°C for two hours and then allowed to cool slowly overnight.

The precipitate was filtered through a 11 cm. Whatman No. 42 filter paper and washed thoroughly with cold water. The filter paper was put in a tared crucible, dried in an oven and ignited in a muffle at a temperature of 500°C. The residue was treated with one drop of concentrated HNO_3, fumed off carefully and reignited at 500°C. The crucible was cooled and weighed. The weight of the precipitate obtained was multiplied by 0.137 to obtain the amount of sulphur.

Manganese:

The method is a combination of a modified form of that of Coleman and Gilbert (1939) and that of Richards M.B. (1930).

Procedure:

About 5 g. of the material were ashed at a temperature of 500°C in a silica basin. The residue was moistened with water, treated with a little concentrated HCl and evaporated to dryness. A few ml. H_2SO_4 (33% by volume) and 3 drops of concentrated HNO_3 were added and the whole evaporated to dryness in a water bath, finishing on a sand bath with final gentle ignition with a bunsen flame. To the residue were added 2-2.5 ml. of 33% H_2SO_4 and a little water and the mixture was evaporated to white fuming stage to remove all traces of chloride.

After cooling the residue was diluted with water and filtered into a 100 ml. test tube to give a volume of about 60 ml./

60 ml. After cooling 10 ml. of concentrated H_2SO_4, 3 ml. of phosphoric acid (85%) and 0.3 gram of potassium periodate were added. The tube was placed in a boiling water bath for about half an hour to develop the full colour of $KMnO_4$, cooled and transferred to a 100 ml. flask and the volume made up. The solutions were then compared in an EEL Colorimeter against standard solutions.

A stock solution may be prepared by dissolving 0.2878 g. of $KMnO_4$ in 250 ml. water in a litre volumetric flask, adding 20 ml. concentrated H_2SO_4 and sodium metabisulphite solution slowly until the solution is just colourless. Any excess of SO_2 is removed by addition of a few drops of HNO_3. Dilute to 1 litre (1 ml. = 1 mg. Mn). To prepare a standard solution put a known amount of this solution of manganous sulphate (10 ml.) into a 100 ml. volumetric flask add 10 ml. H_2SO_4 and 3 ml. phosphoric acid. Dilute to about 60 ml. add 0.3 gm. potassium periodate and proceed as above. When the colour is fully developed, cool and make to volume.

A standard graph was drawn for different standard solutions against the reading in the EEL Colorimeter. This graph was used as standard. At each estimation of manganese a standard solution was prepared and the manganese estimated in it to check the sensitivity of the colorimeter as well as the strength of the standard solution.

Sodium and Potassium:

The method is that of Peach and Tracey (1956).

About/

About 1 gram of the material was ashed at 480°C in a silica basin, cooled, 50 ml. of 10% HCl (by volume) were added, digested on a water bath for about half an hour and filtered through No. 42 Whatman filter paper into a 250 ml. flask. The residue and filter paper were washed with hot water. The filtrate was cooled and made up to 250 ml., 50 ml. were transferred to a 100 ml. flask and made up to volume. The amount of HCl was carefully measured and the digestion controlled so that the final extract contained 1% HCl (by volume). The solution was then compared in the flame photometer against standard solutions of KCl and NaCl containing exactly the same amount of HCl.

Two graphs were drawn, one for Na and the other for K, from appropriate standard solutions. The photometric readings gave directly the contents of these elements from the graphs.

Standard solution of Na and K

Sodium -

Pure "Analar" quality sodium chloride was heated to about 500°C in a furnace for about half an hour, cooled and 0.634 gram weighed into a 500 ml. volumetric flask. It was dissolved and made up to the mark with 1% HCl (by volume). To make a solution of 1 milligram of Na/100 ml., the dilution of the above stock solution was 1:50 in 1% HCl.

Potassium -

0.477 g. of dry "Analar" quality potassium chloride was weighed/

weighed, transferred into a 500 ml. volumetric flask, dissolved and made up to the mark with 1% HCl. For the standard solution of 10 milligram/100ml. the stock solution was diluted 10:50.

Total Nitrogen -

The estimation was according to the usual method of Kjeldhal and the catalytic agent was a mixture of selenium and potassium sulphate.

Albuminoid Nitrogen(Protein nitrogen) -

The method was that recommended in Official Methods of Analysis (1955) by A.O.A.C.

Reagent:-

Cupric hydroxide. Dissolve 100 g. of $CuSO_4,5H_2O$ in 5 litres of water, add 2.5 ml. of glycerol and then add 10% solution of NaOH until the solution is slightly alkaline. Filter, rub the precipitate an a mortar with water containing 5 ml. of glycerine per litre and wash by decantation or filtration until washings are no longer alkaline. Again rub the precipitate in the mortar with water containing 10% glycerol, thus preparing a uniform gelatinous mass that can be measured with a pipette. Determine approximately the quantity of $Cu(OH)_2$ in 50ml. by diluting to 50 ml. with water, filtering, washing, igniting and weighing as CuO.

Determination:-

About 1.0 g. of the sample was placed in a conical beaker and 100 ml. of boiling water were added and the mixture boiled/

boiled for 30 minutes on a water bath. A quantity of the reagent containing about 0.5 g. of $Cu(OH)_2$ was added. The mixture was stirred thoroughly, filtered when cold and, without removing the precipitate from filter, the N was determined as in Kjeldahl method. If material (such as seeds, seed residue or oil cake) is rich in alkaline phosphate, 1-2 ml. of 10% NH_3 - free $K(Al(SO_4)_2,12\ H_2O$ is added to decompose them, then $Cu(OH)_2$ and mixed well by stirring. If this is not done $Cu_3(PO_4)_2$ and free alkali may be formed and protein-Cu precipitate partially dissolved in the alkaline liquid.

Reproducibility of the different methods of chemical analysis

	Ca	Mg	Cl	P	S	Na	K	Mn
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Sample I	6.47	6.10	16.0	10.15	7.65	4.30	36.80	1.46
*Added elements	80.08	120.64	44.37	59.60	92.08	2.50	20.00	1.50
Total	86.55	126.74	60.37	69.75	99.73	6.80	56.80	2.96
Recovery	87.00	124.80	59.50	69.81	98.85	6.70	56.45	2.90
% Recovery	100.5	98.5	98.6	100.1	99.2	98.5	99.4	98.0
Sample II	9.51	6.83	12.02	11.63	6.94	2.47	35.86	0.90
*Added elements	12.01	18.10	29.22	2.98	46.59	3.00	10.00	1.00
Total	21.52	24.93	41.24	14.61	53.53	5.47	45.86	1.90
Recovery	21.50	24.84	41.18	14.59	52.95	5.36	45.63	1.86
% Recovery	99.9	99.6	99.9	99.9	98.9	98.0	99.5	97.9
Sample III	9.97	4.33	8.70	11.54	5.24	1.44	29.44	1.43
*Added elements	24.02	24.30	58.45	29.80	28.18	1.50	5.00	2.00
Total	33.99	28.63	67.15	41.34	33.42	2.94	34.44	3.43
Recovery	33.91	28.48	66.85	41.05	32.93	2.92	33.85	3.38
% Recovery	99.8	99.4	99.6	99.3	98.5	99.3	98.3	98.5

* The elements were added as the following Analar compounds:-

CaO; MgO; NaCl; KH_2PO_4; K_2SO_4; NaCl; KCl; $MnSO_4.4H_2O$.

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